SUPPLEMENT 3

HONG KONG MEDICAL JOURNAL 香港醫學雜誌 24(S3) Volume 24 Number 3 June 2018

HONG KONG

Volume 24 Number 3 **June 2018**

The official publication of the
Hong Kong Academy of Medicine and
the Hong Kong Medical Association

MEDICAL JOURNAL
香港醫學雜誌

Health and Medical Research Fund

Research Dissemination Reports

醫療衞生研究基金

研究成果報告

Clinical Genetics
臨床遺傳學

Neurology
神經病學

Reference Values, Biomarkers and Diagnostics
參考值、生物標誌和診斷

Supplement 3

ISSN 1024-2708

香港醫學專科學院出版社
HONG KONG ACADEMY OF MEDICINE PRESS

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Volume 24 Number 3 **June 2018**
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Health and Medical Research Fund

Research Dissemination Reports

REFERENCE VALUES, BIOMARKERS AND DIAGNOSTICS

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund (and its predecessor funds) administered by the Food and Health Bureau. In this edition, we present 13 dissemination reports of projects related to clinical genetics, neurology, and reference values, biomarkers and diagnostics. In particular, three projects are highlighted due to their potentially significant findings, impact on healthcare delivery and practice, and/or contribution to health policy formulation in Hong Kong.

In Hong Kong, breast cancer accounts for about one-third of all newly diagnosed cancers and about 11% of all cancer deaths. Hereditary breast and ovarian cancer syndrome is a genetic disease in which alterations in *BRCA1* and *BRCA2* genes are common. About 10% of breast cancer cases in Hong Kong are inherited. Kwong et al[1] screened over 630 blood samples obtained from local Chinese breast, ovarian, and prostate cancer patients with a panel targeting 25 *BRCA* mutations. The prevalence of *BRCA* mutations among various local cancers was determined. Development of a screening panel for recurrent mutations offers a simple, rapid, and affordable routine molecular diagnostic method for prevention or management of these high-risk patients and their families with BRCA mutations.

Post-stroke apathy is a debilitating condition with a prevalence of 20% to 25% in stroke patients. Post-stroke apathy is often undiagnosed and thus untreated, even though it may impair stroke recovery. Tang et al[2] aimed to evaluate the clinical and magnetic resonance imaging correlates of post-stroke apathy in a cohort of stroke survivors, and to describe the 12-month course of post-stroke apathy in over 260 local Chinese stroke patients. They found that the prevalence of post-stroke apathy at 3 months was 24.7%, with older age, male sex, history of hyperlipidaemia, depressive symptoms, a lower level of cognitive function, and functional disability identified as risk factors. A pontine acute infarct on magnetic resonance images was an independent predictor of post-stroke apathy at 3 months. The authors stressed the psychological burden of post-stroke apathy and thus early identification and treatment are essential.

Colorectal cancer is the most common cancer in Hong Kong and surgery is the only curative treatment. Adjuvant chemotherapy is indicated in high-risk stage II and stage III disease to reduce the risk of recurrence and metastasis after surgery. For stage IV disease, chemotherapy is the mainstay of treatment for palliation. Capecitabine is often used as an oral chemotherapeutic agent. During capecitabine treatment, the rate of toxicity differs between western and Asian patients. A higher serum folate level has been associated with a higher rate of toxicity in western populations. Chan et al[3] conducted a prospective study to examine the association of serum folate level with toxicity during capecitabine treatment in over 140 local Chinese patients. They found that a higher serum folate level is associated with a higher rate of moderate-to-severe toxicity of capecitabine in patients with colorectal cancer.

We hope you will enjoy this selection of research dissemination reports. Electronic copies of these dissemination reports and the corresponding full reports can be downloaded individually from the Research Fund Secretariat website (https://rfs2.fhb.gov.hk/). Researchers interested in the funds administered by the Food and Health Bureau also may visit the website for detailed information about application procedures.

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Screening for founder and recurrent BRCA mutations in Hong Kong and US Chinese populations

A Kwong*, VY Shin, ESK Ma, CTL Chan, JM Ford, AW Kurian, E Tai

KEY MESSAGES

1. A total of 637 blood samples (441 breast, 155 ovarian, and 41 prostate cancers) were obtained in a local Chinese population.
2. The overall prevalence of *BRCA* mutation was 8.05% and the pickup rate of the recurrent panel was 3.52%. Nearly half of the mutations were covered by this panel.
3. We identified three *BRCA* mutations that were seen only in patients with ovarian cancer.
4. Of 79 Chinese breast cancer samples collected from overseas, two recurrent mutations were identified.
5. We compared 84 known mutation cases from overseas (comprising 62 different types of mutations) with our recurrent spectrum. Of which, 15 have been identified in Hong Kong and seven of them were covered in the recurrent panel.

Hong Kong Med J 2018;24(Suppl 3):S4-6
HMRF project number: 01121376

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This summary is based on studies first reported in:
(1) Kwong A, Ho JC, Shin VY, et al. Rapid detection of *BRCA1/2* recurrent mutations in Chinese breast and ovarian cancer patients with multiplex SNaPshot genotyping panels. Oncotarget 2017;9:7832-43.
(2) Kwong A, Shin VY, Au CH, et al. Detection of germline mutation in hereditary breast and/or ovarian cancers by next-generation sequencing on a four-gene panel. J Mol Diagn 2016;18:580-94.
(3) Kwong A, Shin VY, Cheuk IW, et al. Germline RECQL mutations in high risk Chinese breast cancer patients. Breast Cancer Res Treat 2016;157:211-5.

Introduction

In Hong Kong, breast cancer accounts for approximately one-third of all newly diagnosed cancers and 11.1% of all cancer deaths. An exponential increase in the incidence is expected in Hong Kong and other Asian regions, in particular China. Hereditary breast and ovarian cancer (HBOC) syndrome is a genetic disease in which alterations in *BRCA1* and *BRCA2* genes are common. About 10% of breast cancer cases in Hong Kong are inherited.[1] Patients with a *BRCA* mutation and their family members have a higher risk of developing breast cancer (45%-65%) or ovarian cancer (11%-39%) by the age of 70 years, compared with those without a *BRCA* mutation.[2] Screening and risk-reduction intervention for *BRCA* carriers and their family members are recommended. Identification of ethnic-specific founder and hotspot (recurrent) mutations in Chinese patients would be beneficial for the Chinese population. A genetic testing panel was designed for the population to achieve better risk assessment and preventive measures.

Methods

We recruited high-risk patients who (1) were diagnosed with breast cancer at the age of ≤45 years; (2) had bilateral breast cancer; (3) had triple-negative or medullary type pathology; (4) had ovarian cancer at any age; (5) had male breast cancer; (6) had at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age; and (7) had one relative with a *BRCA* mutation.

Genomic DNA and RNA samples were extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen). In the case of splicing variant analysis in transcript level, RNA samples were reverse transcribed to cDNA samples using Superscript III First Strand Synthesis System (Invitrogen).

A total of 25 hotspot mutations, including founder and recurrent mutations reported in a Chinese ethnic group, were identified through a literature search. A single base extension assay, SNaPshot, was adopted for mutation screening. In brief, all 25 mutations were either amplified by polymerase chain reaction (PCR) or multiplex PCR. Single base extension reaction was performed by the SNaPshot kit. After purification, SNaPshot products were run with LIZ120 size standard in a sequencing analyser. Data were processed by Genescan Analysis. Relative intensity of the two alleles in each sample was calculated to confirm heterozygosity. The signal from the normal control DNA was used as reference.

In patients with founder/recurrent mutations,

further validation was performed by full gene sequencing. Mutation analysis was performed by direct DNA sequencing of all coding exons of *BRCA1* and *BRCA2* and partial flanking intronic sequences. Bi-directional sequencing was performed. Sequencing results were compared with the reference DNA sequences using Variant Reporter software (Applied Biosystems) and then reviewed manually.

The Fluidigm Access Array System (Fluidigm, San Francisco [CA], USA) was used to generate separate pools of 74 PCR amplicons per sample to target all exons of the *BRCA* genes plus 10 bp from intron-exon boundaries. Dual 8-bp barcode nucleotide sequences were incorporated in the ends of each amplicon for sample identification. Paired-end sequencing of the amplicons (2 x 300bp) was performed on a MiSeq (Illumina, San Diego [CA], USA) with reagent kit v3.

Results

All patients underwent *BRCA* screening with the recurrent panel covering 25 loci. Those who tested negative were then subjected to amplicon-based next-generation sequencing (NGS). The prevalence of *BRCA* mutations among breast cancer patients was 7.94% (35/441) and the pickup rate of the recurrent panel was 3.4% (15/441). Among ovarian cancer patients, *BRCA* mutations were identified in 8.39% (13/155), and the pickup rate of the recurrent panel was 3.87% (6/155). Overall, the prevalence of *BRCA* mutation in the local Chinese cohort was 8.05% (48/596), and the pickup rate of the recurrent panel was 3.52%. Interestingly, we identified three *BRCA1* mutations (c.4046, c.212+3A>G, and c.5335delC) by NGS, which were seen only in those with ovarian cancer, not breast cancer.

In 79 blood samples collected overseas, we identified two recurrent mutations (*BRCA2* c.3109C>T and *BRCA2* c.4965delC) using the recurrent panel and a novel *BRCA2* c.3165_3167delinsCC mutation by NGS. Additionally, we compared the spectra of *BRCA* mutations between Hong Kong and overseas. We received 84 Chinese breast cancer cases that covered 62 different types of mutation (20 *BRCA1* and 42 *BRCA2*). In addition, only 15 types of mutations had been previously identified in Hong Kong. Our recurrent panel covered seven types of these mutations (2 *BRCA1* and 5 *BRCA2*) and was expected to pick up 17 of the 84 cases. The most predominant mutations in this overseas cohort were *BRCA2* c.7878G>A and c.5164_5165delAG.

Discussion

In this pilot study, <10% of the Chinese patients harboured *BRCA1* or *BRCA2* mutations, and nearly half of the mutations were recurrent. Consistent with our previous findings,[1] this screening method using the recurrent panel could detect 43.8% (21/48) of all *BRCA* mutations in patients with HBOC. Furthermore, *BRCA1* [c.964delG (n=3); c.4372C>T (n=3)] and *BRCA2* c.3109C>T (n=7) were the most common mutations in the local cohort. *BRCA2* predominance is common among Chinese patients.

The spectrum of *BRCA* mutation varies across ethnicities, and its prevalence and dominancy also varies among different populations.[3] The cost of genetic testing and lack of coverage by the local healthcare system are barriers to mutation screening in Asia and in Hong Kong.[3] Development of a screening panel for recurrent mutations offers a simple, rapid, and affordable routine molecular diagnostic method for prevention or management of these high-risk patients and their families with *BRCA* mutations.[4]

The bioinformatics and sequencing data analysis of NGS remain challenging, with diverse analysis tools and databases available. Development of a breast cancer genetic screening strategy for Chinese ethnicity could benefit from this new approach and improve cancer risk assessment and management for patients with HBOC.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01121376). We thank John Ho, Isabella Cheuk, Jiawei Chen, Jennifer Siu, Tommy Au, Fian Law, Donna Ip, Wing Pan Luk, Ling Hui Fung, and Cecilia Ho for their technical support and data analysis. We also thank all the doctors and nurses from the breast cancer research groups in the departments of surgery and oncology of the contributing hospitals in Hong Kong for patient recruitment. We also thank Laura Esserman and Beth Crawford (University of California), Jeffrey Weitzel (City of Hope Cancer Centre), Edmund Tai (Palo Alto Medical Foundation), Mike Field (Royal North Shore Hospital), Susan Domchek (University of Pennsylvania), James Ford and Allison Kurian (Stanford University) and Chiun-sheng Huang (National Taiwan University Hospital) for providing samples and mutation spectrum of Chinese patients with breast cancer.

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Genetic screening for familial hypercholesterolaemia in Hong Kong

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KEY MESSAGES

1. Cascade screening of family members of known index cases is an effective approach to identify new cases of familial hypercholesterolaemia (FH).
2. Family screening is carried out using a combination of plasma lipid profiling and genetic testing. If the causative mutation is unknown or if genetic testing is unavailable, screening can be performed using plasma lipid profiling alone.
3. Over 90% of the FH subjects with a pre-treatment low-density lipoprotein cholesterol level of >8 mmol/L had an identifiable genetic cause.
4. Causative mutations in most FH patients were found in the low-density lipoprotein receptor gene.

Hong Kong Med J 2018;24(Suppl 3):S7-10
HMRF project number: 01121256

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Introduction

Familial hypercholesterolaemia (FH) is an inherited disorder of lipid metabolism that results in high levels of low-density lipoprotein cholesterol (LDL-C) and increased risk of premature cardiovascular disease. The major genetic causes are mutations in the LDL-receptor (*LDLR*) gene, the apolipoprotein B-100 (*APOB*) gene, and/or the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene. Recent studies suggest that heterozygous FH is more common than previously thought and affects approximately 1 in 200 to 250 persons in western countries. Early diagnosis and treatment with statin significantly improves the prognosis and reduces the cardiovascular burden.

Diagnosis of FH is mainly based on lipid levels and family history. Clinical diagnostic tools have been developed to standardise the phenotypic diagnosis of FH. The most widely used scoring systems are the Dutch Lipid Clinic Network (DLCN) Criteria and the Simon Broome Criteria.[1] These systems have various predictive values depending on the test population but have inherent limitations in sensitivity and specificity. Recent guidelines recommend that all patients with clinical and biochemical features of FH undergo DNA testing to confirm the diagnosis, and genetic cascade family screening enables unambiguous identification of affected relatives.[2,3]

Genetic testing for FH is not available in the public health care system in Hong Kong so the diagnosis of FH is based on clinical evidence. Family screening is performed only on an ad-hoc basis because of limited staffing and resources. It is likely that a considerable proportion of patients with FH remain undiagnosed. This study aimed to implement systematic cascade genetic screening of FH patients and their families. The study was expected to provide information about the spectrum of genetic mutations in Hong Kong Chinese patients with FH.

Methods

Index patients with a clinical diagnosis of FH and/or severe hypercholesterolaemia were recruited from the Queen Mary Hospital, Ruttonjee Hospital, and Pamela Youde Nethersole Eastern Hospital. The diagnosis of FH was classified as definite, probable, or possible according to the DLCN Criteria. Genetic analysis was carried out in index cases, and first-degree relatives were invited to attend cascade screening to detect potential new cases based on genetic testing (when a family-specific causative variant had been identified) or from phenotypic diagnostics. The newly confirmed cases were considered new index cases and their first-degree relatives were subsequently screened whenever possible.

Genomic DNA was isolated from peripheral blood leukocytes, and mutations were identified by Sanger sequencing of the coding regions of *LDLR*, *APOB*, or *PCSK9* genes. Exons and their adjacent introns, 5'-UTR and 3'-UTR, were amplified by polymerase chain reaction, and standard sequencing in both forward and reverse directions was performed using the 3730xl DNA Analyzer (Applied Biosystems, Foster City [CA], United States) at the Centre for Genomic Sciences of The

University of Hong Kong. Pathogenicity of identified variants was assessed by reference to published data in the following databases: the University College London FH mutation database (http://www.ucl.ac.uk/ldlr/Current/), the Human Gene Mutation Database (http://www.biobase2international.com/product/hgmd), and Ensembl GRCh38. Pathological assessment of novel variants was performed using multiple online tools.

TABLE 1. Clinical characteristics and pre-treatment lipid levels of index familial hypercholesterolaemia patients

Characteristic	Male (n=42)*	Female (n=52)*
Age, y	50.2±11.9	53.2±10.3
Weight, kg	70.3±9.5	58.9±8.4
Body mass index, kg/m^2	24.6±5.7	24.8±3.2
Cardiovascular disease	43%	40%
Total cholesterol, mmol/L	9.3±1.9	9.7±2.3
Total triglycerides, mmol/L	1.6±0.8	1.9±0.8
Low-density lipoprotein cholesterol, mmol/L	7.6±1.9	7.8±2.2
High-density lipoprotein cholesterol, mmol/L	1.2±0.4	1.4±0.5
Xanthoma/xanthelaesma at diagnosis	21%	15%

* Data are presented as mean ± standard deviation or % of patients

TABLE 2. Types of mutations in familial hypercholesterolaemia index patients

Types of mutations	Total No.	Low-density lipoprotein cholesterol, mmol/L*	No. of unique mutations	No. of novel mutations
Single mutation				
LDLR (total)	51	8.0±1.5	29	10
Splicing	2	9.0±0.4	1	0
Frameshift	2	8.2±1.3	2	1
Nonsense	4	8.5±1.6	3	1
Missense	43	7.9±1.6	23	8
APOB (missense)	3	7.3±1.1	2	0
PCSK9	0	-	0	0
Two mutations				
LDLR + *LDLR*	5	10.4±4.5	4	2
LDLR + *APOB*	3	9.6±3.0	3	0

* Data are presented as mean ± standard deviation

TABLE 3. Proportions of mutation-positive patients according to low-density lipoprotein cholesterol level

Low-density lipoprotein cholesterol, mmol/L	Total No.	Proportion with mutation (%)
<6	18	39
6-6.9	16	44
7-7.9	22	68
8-8.9	16	94
9-9.9	15	93
>10	7	71

Results

A total of 98 index patients were recruited and 94 probands were identified. Four patients were found to be related (Table 1). All but two patients were taking statins and 55 patients were taking two or more lipid-lowering agents, and two were receiving plasmapheresis. Only 20 patients who were undergoing treatment had an LDL-C level of <2.6 mmol/L, and seven of these patients had an LDL-C level of <1.8 mmol/L.

Genetic analysis was carried out in the 94 unrelated probands. Definite or likely pathogenic mutations were identified in 62 patients (Table 2). Most mutation-positive patients had a heterozygous *LDLR* gene mutation. There were five cases of compound heterozygous mutation, three cases of double heterozygous mutation, and one case of homozygous mutation of c.1474G>A in exon 10. Overall, mutation-positive patients had a significantly higher mean ± standard deviation LDL-C level at diagnosis than mutation-negative patients (8.1±1.9 vs 6.4±1.4 mmol/L, P<0.01). There was a stepwise increase in the proportion of patients with causative mutations identified according to the LDL-C stratum (Table 3). Of patients with an LDL-C level of >8 mmol/L, 90% were mutation-positive.

As genetic testing is not available in the public health care system in Hong Kong, diagnosis of FH is based on phenotypic criteria. We compared the diagnostic performance of the DLCN Criteria with the modified DLCN Criteria for Chinese, which uses lower LDL-C cut-offs based on population data derived in China.[4] On validation against genetic testing, the DLCN Criteria demonstrated the best overall performance in diagnosing FH (82.8% sensitivity, 53.3% specificity, 79.1% positive predictive value, and 59.3% negative predictive value). The modified DLCN Criteria had high sensitivity (93.8%) but low specificity (26.7%). The Simon Broome Criteria had lower sensitivity (64.0%) and higher specificity (56.6%), compared with the DLCN Criteria.

Cascade family screening was offered to all probands. Relatives of 45 of the 62 probands with causative mutations identified (167 first- and second-degree relatives) attended for screening. In contrast, relatives of only 13 of 32 probands with no mutations were identified (36 first-degree relatives) attended for screening. Only adults were screened, as screening for children and adolescents has not been approved. Of 203 relatives who attended for screening, 122 were identified to have FH: 48

were newly diagnosed and 74 were aware of having hypercholesterolaemia, although 53% of the latter had never been treated or had stopped treatment. The diagnosis of FH was excluded in 81 patients. In family members identified to have FH, 81% were diagnosed based on the finding of a causative mutation on genetic testing. The remaining were diagnosed based on clinical phenotype in their pedigrees when genetic testing did not detect a causative mutation.

Discussion

Our findings are similar to those previously reported in Chinese and Caucasian populations—namely, that the causative mutations in most FH patients are in the *LDLR* gene. Although the prevalence varies in different populations, heterozygous *LDLR*, *APOB*, and *PCSK9* mutations are found in >90%, ~5%, ~1%, respectively, of heterozygous FH patients with a causative mutation. We identified one homozygous FH patient with the mutation c.1474G>A in exon 10 of *LDLR*. This mutation causes a defective protein and leads to reduced function of *LDLR* rather than a null mutation.[5] We also identified a number of compound heterozygotes and double heterozygotes. These individuals have higher LDL-C levels, and such genotypes have been reported in some cases of phenotypic homozygous FH.

No genetic cause was identified in 34% of our FH patients, consistent with the proportion reported in the literature. This may be due to a number of reasons. We did not investigate rare mutations in minor genes, such as *APOE*, *ABCG5*, *ABCG8*, *LIPA*, or *STAP1*, which can phenotypically resemble FH. There may have been undiscovered monogenic causes of hypercholesterolaemia in some patients, and some patients may have had a polygenic cause and carried a disproportionately high burden of multiple small-effect common variants that raised the plasma LDL-C level. Other possible causes include epigenetic effects and interaction of environmental factors with unknown genetic determinants.

LDL-C is the most discriminating factor in the diagnosis of FH. Sensitivity and specificity of any scoring criteria can be varied by changing the LDL-C cut-off value. Our data suggest that LDL-C cut-offs in the DLCN Criteria can be applied to the Hong Kong population to diagnose FH and select patients for genetic testing. For genetic testing based on LDL-C levels alone, our data show a cut-off of 8 mmol/L, as 90% of the index subjects with a LDL-C level of >8 mmol/L had an identifiable genetic cause.

The most cost-effective approach to identify new FH patients is cascade screening of family members of known index cases. Having an identifiable genetic cause increases the likelihood of family members agreeing to participate in screening. Overall, the awareness of FH in family members is low in Hong Kong and the high risk of cardiovascular disease secondary to FH is poorly recognised. Among the relatives of FH patients who knew they had a high cholesterol level from previous lipid testing, half had never been treated or had discontinued treatment, especially those with a milder phenotype. There is a need to increase awareness and understanding of the condition to better prevent the development of cardiovascular disease in these patients.

Limitations

Our study has several limitations. We did not investigate rare mutations in minor genes, and we did not evaluate the potential contribution of polygenic effects. Pathological assessment of novel variants was based on in silico analysis only; further functional studies are needed to confirm the pathogenic effects. Only adults were screened; screening of relatives younger than 18 years was restricted.

Conclusions

In clinically ascertained patients with FH and/or severe hypercholesterolaemia, about two-thirds had a discrete genetic basis of disease, with most causative mutations occurring in the *LDLR* gene. Genetic cascade screening is feasible. Having an identifiable genetic cause increases the likelihood of family members agreeing to participate in screening.

Familial hypercholesterolaemia is underdiagnosed and undertreated in Hong Kong. Awareness of the condition in relatives of affected individuals and in the community is low, and education of the public and health care professionals is needed. Cascade screening of family members is an effective means to identify new FH cases. Screening can be carried out using a combination of plasma lipid profiling and genetic testing. If the causative mutation is unknown or genetic testing is unavailable, screening can be performed using plasma lipid profiling alone. Data collected in this study may help formulate future health care policies on the eligibility of FH patients for novel therapies such as PCSK9 inhibitors. About 90% of our FH patients with a LDL-C level of >8 mmol/L had an identifiable genetic cause. These patients with severe FH are unlikely to achieve the recommended LDL-C target with current oral lipid-lowering agents. They are candidates for PCSK9 inhibitors if funding can be obtained.

Acknowledgement

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01121256).

Ethical Approval

The study was approved by the Institutional Review

Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster and Hong Kong East Cluster Research Ethics Committee (UW 12-494 and HKEC-2013-005, respectively). Informed consent was obtained from each participant.

Declaration

The authors have no conflict of interest to disclose.

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Target-enriched massively parallel sequencing for genetic diagnosis of hereditary hearing loss in patients with normal array CGH result

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KEY MESSAGES

1. In our cohort, 15 common hearing-loss mutations with a high carrier frequency (15.9%) were screened; *GJB2* c.109G>A was the most common mutation (10.9%).
2. For patients with hearing loss who were negative for the 15 common mutations, our hearing-loss target capture panel combined with a massively parallel sequencing approach increased detection of pathogenic mutations or likely pathogenic variants by 21%.

Hong Kong Med J 2018;24(Suppl 3):S11-4
HMRF project number: 01120256

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This summary is based on studies first reported in: (1) Chen Y, Cao Y, Li HB, et al. SNaPshot reveals high mutation and carrier frequencies of 15 common hearing loss mutants in a Chinese newborn cohort. Clin Genet 2015;87:467-72. (2) Lu Y, Zhou X, Jin Z, et al. Resolving the genetic heterogeneity of prelingual hearing loss within one family: Performance comparison and application of two targeted next generation sequencing approaches. J Hum Genet 2014;59:599-607.

Introduction

Hearing loss is defined as a partial or total inability to hear sound in one or both ears. It affects 278 million people worldwide and is the most common birth defect.[1] It has been estimated that the incidence of bilateral permanent sensorineural hearing loss (≥40 dB) is 1 per 500 infants at birth.[2] Genetic causes of hearing loss are estimated to account for as many as 68% of cases in newborns and 55% of cases by the age of 4 years. Up to 70% of hereditary hearing-loss cases are non-syndromic, whereas 30% are syndromic. To date, at least 75 non-syndromic deafness genes and more than 1000 discrete deafness-causing mutations have been described. For example, connexin 26 (encoded by *GJB2*) is responsible for more than half of the cases of hereditary pre-lingual sensorineural hearing loss in many populations, by virtue of hotspot mutations, as well as founder mutations among different ethnic groups. Mutations in pendrin (encoded by *SLC26A4*) can cause both non-syndromic deafness (DFNA4, MIM 600791) and syndromic deafness (Pendred syndrome, MIM 274600). Hereditary hearing loss is extremely heterogeneous.

In Hong Kong, the Department of Health offers genetic diagnosis of hearing loss for four genes only: *GJB2*, *GJB6*, mitochondrial 12S ribosomal RNA gene (1555A>G mutation), and *PAX3*. There is a strong need for a comprehensive, robust, and cost-effective method to enable genetic diagnosis of hearing loss. Children with hearing loss identified before 6 months of age who begin appropriate interventions demonstrate superior language skills to those identified after 6 months of age. Early identification of mutations can inform medical care and improve prognosis—for example, avoidance of ototoxity from aminoglycoside antibiotic therapy in the presence of the mitochondrial 1555A>G mutation.

Methods

This study aimed to estimate the frequency of 15 well-known pathogenic mutations in a sample of the Chinese population, based on the screening of a neonatal cohort in Suzhou, China. A total of 5800 newborns (3077 males and 2723 females) were enrolled between October 2011 and February 2012 at the Suzhou Hospital. To compare results with the general population of China, these blood samples were obtained from an established nationwide screening programme of inborn errors of metabolism, without special selection.

To screen for common hearing-loss mutations, 15 mutations were detected by massively parallel sequencing with the SNaPshot Multiplex System (Thermo Fisher Scientific, Waltham [MA], United States). The screening test covered the following mutations: 35delG, 109G>A, 176-191del16, 235delC, and 299-300delAT in *GJB2*; c.919-2A>G, 1174A>T, 1229C>T, 2027T>A, and 2168A>G in *SLC26A4*; and mt1494C>T, mt1555A>G, mt3243A>G, mt7444G>A, and mt7445A>G in the mitochondrial genome. In the target enrichment step, the targeted

regions of interest were designed to cover all the exons and flanking 15 bp of 261 human genes that are known to be causative hearing-loss genes or candidates. A solution-based capture approach was used (NimbleGen SeqCap EZ Choice kit; Roche, Basel, Switzerland).

For bioinformatics analysis, data were processed in the following sequence. (1) Raw data were generated using the Illumina Pipeline (version 1.3.4; https://www.illumina.com/informatics/infrastructure-pipeline-setup.html). (2) Clean reads were selected and unqualified sequences were removed from the raw data using a local dynamic programming algorithm, and (3) reads were aligned against the reference human genome from the National Center for Biotechnology Information database (HomoloGene Build 37; https://www.ncbi.nlm.nih.gov/homologene) using the Burrows Wheeler Aligner Multi-Vision software package (http://bio-bwa.sourceforge.net/). (4) Single-nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs) were identified using the SOAPsnp (Short Oligonucleotide Analysis Package; http://soap.genomics.org.cn/soapsnp.html) and the GATK Indel Genotyper (Genome Analysis Toolkit; http://www.broadinstitute.org/gsa/wiki/index.php/), respectively. (5) Sequence variations were annotated using an in-house pipeline, consisting of the gene annotation software Reference Sequence (RefSeq; https://www.ncbi.nlm.nih.gov/RefSeq). (6) Known polymorphisms and minor alleles were identified with the dbSNP138 SNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi?view+summary=view+summary&build_id=138), HapMap database (https://www.ncbi.nlm.nih.gov/probe/docs/projhapmap/), 1000 Genomes database (http://www.internationalgenome.org/), and Exome Aggregation Consortium database (http://exac.broadinstitute.org/). (7) Variants (exonic, splice site, silent, missense) were then characterised. (8) Biological function was then predicted, including whether an amino acid substitution would affect protein function and thus be prioritised for further study, using tools for sorting intolerant from tolerant (SIFT; http://sift.jcvi.org/) and polymorphism phenotyping (PolyPhen; http://genetics.bwh.harvard.edu/pph2/), as well as other third-party software.

Results

In this cohort, 15.9% (923/5800) of newborns carried at least one of the 15 mutations of interest (Table 1), indicating that 1 in 6.3 newborns carried at least one mutant allele of a hearing-loss gene. The *GJB2* mutants accounted for up to 12.7% (735/5800) of cases. Additionally, 2.17% (126/5800) of newborns had at least one mutant allele of *SLC26A4*, and 1.07% (62/5800) were carriers of the mitochondrial mutant allele. The most prevalent mutated allele was *GJB2* c.109G>A, which had an allele frequency of 5.26% (610/11600), followed by *GJB2* c.235delC (0.94%, 109/11600) and *SLC26A* c.919-2A>G (0.84%, 98/11600). These alleles had a carrier frequency of approximately 10.29% (597/5800), 1.88% (109/5800), and 1.62% (94/5800), respectively.

TABLE 1. Carrier frequency of 15 common mutations

Mutations	No. of individuals (n=5800)	Carrier frequency (%)
Total	923	15.914
GJB2 c.109G>A	597	10.290
GJB2 c.235delC	109	1.879
SLC26A4 c.919-2A>G	94	1.621
mt7444G>A	41	0.707
SLC26A4 c.2168A>G	20	0.350
GJB2 c.299-300delAT	17	0.293
GJB2 c.176-191del16bp	11	0.190
SLC26A4 c.1174A>T	9	0.156
mt3243A>G	9	0.155
mt1555A>G	8	0.138
mt7445A>G	3	0.052
SLC26A4 c.2027T>A	2	0.034
GJB2 c.35delG	1	0.017
SLC26A4 c.1229C>T	1	0.017
mt1494C>T	1	0.017

In this cohort, 0.48% (28/5800) of newborns were genetically diagnosed with hearing loss because of mutations in *GJB2* and *SLC26A4*, whereas 19 newborns carried homozygous mutations in *GJB2* or *SLC26A4*. Nine newborns carried compound *GJB2* mutations, of whom eight harboured a *GJB2* c.109G>A mutation and a second mutation. Seven of these 28 newborns failed the otoacoustic emission (OAE) test for at least one ear. All other newborns passed either the initial OAE screening or the 1-month OAE follow-up test. No newborn carrying only one mutant allele failed both OAE tests. Genotypic and phenotypic information about these samples are provided in Table 2. Interestingly, newborns who carried homozygous *GJB2* c.109G>A mutations showed varied phenotypes, from a normal result to bilateral failure of the OAE test.

To determine the clinical application of a target-enriched massively parallel sequencing system as a comprehensive, robust next-generation genetic test for hereditary hearing loss, a total of 100 patients with hearing loss (including 42 cases from 24 hearing-loss families) and with normal

TABLE 2. Genetically diagnosed cases of hearing loss

Mutation identified	Genotype	No. of cases	No. of cases failed otoacoustic emission test
Total		28	7 (25%)
GJB2 c.109G>A	Homozygous	13	4
GJB2 c.109G>A + *GJB2* c.176-191del16	Compound heterozygous	1	0
GJB2 c.109G>A + *GJB2* c.235delC	Compound heterozygous	6	2
GJB2 c.109G>A + *GJB2* c.299-300delAT	Compound heterozygous	1	0
GJB2 c.35delG + *GJB2* c.235delC	Compound heterozygous	1	0
SLC26A4 c.919-2A>G	Homozygous	4	1
SLC26A4 c.1174A>T	Homozygous	2	0

array CGH result were recruited, of whom 90% were referred because of congenital or early-onset hearing loss. Two families were suspected to have Pendred syndrome (Fig 1). Overall, read depth for each sample ranged from 86 to 352, with a target region of coverage of between 94.9% and 98.6%.

After primary bioinformatics analysis, an average of 6000 SNPs and INDELs were annotated. Data were filtered conservatively according to an established pipeline with consideration of (1) mean depth and read counts of ≥10, (2) removal of 3'UTR, 5'UTR, downstream, upstream, and non-coding exon transcript variants, and (3) removal of non-coding change types. We also compared the SNP allele frequency in an ethnically matched population in the 1000 Genomes or Exome Aggregation Consortium databases. Twenty-four patients were identified to carry at least one pathogenic or likely-pathogenic mutation in the known hearing-loss genes. Our data confirmed that *GJB2* c.109G>A is a common mutation in the Asian population, with a carrier frequency of as high as 10.9%. Among the 24 patients identified by target-enrichment sequencing, three patients who were known to be homozygous for *GJB2* c.109G>A were referred for further analysis, owing to severe or profound hearing loss. Two patients were found to carry other mutations: *BSND* c.10G>A in one, and *ATP6V0A4* c.1727T>A and *TMPRSS3* c.325C>T in the other (Fig 2).

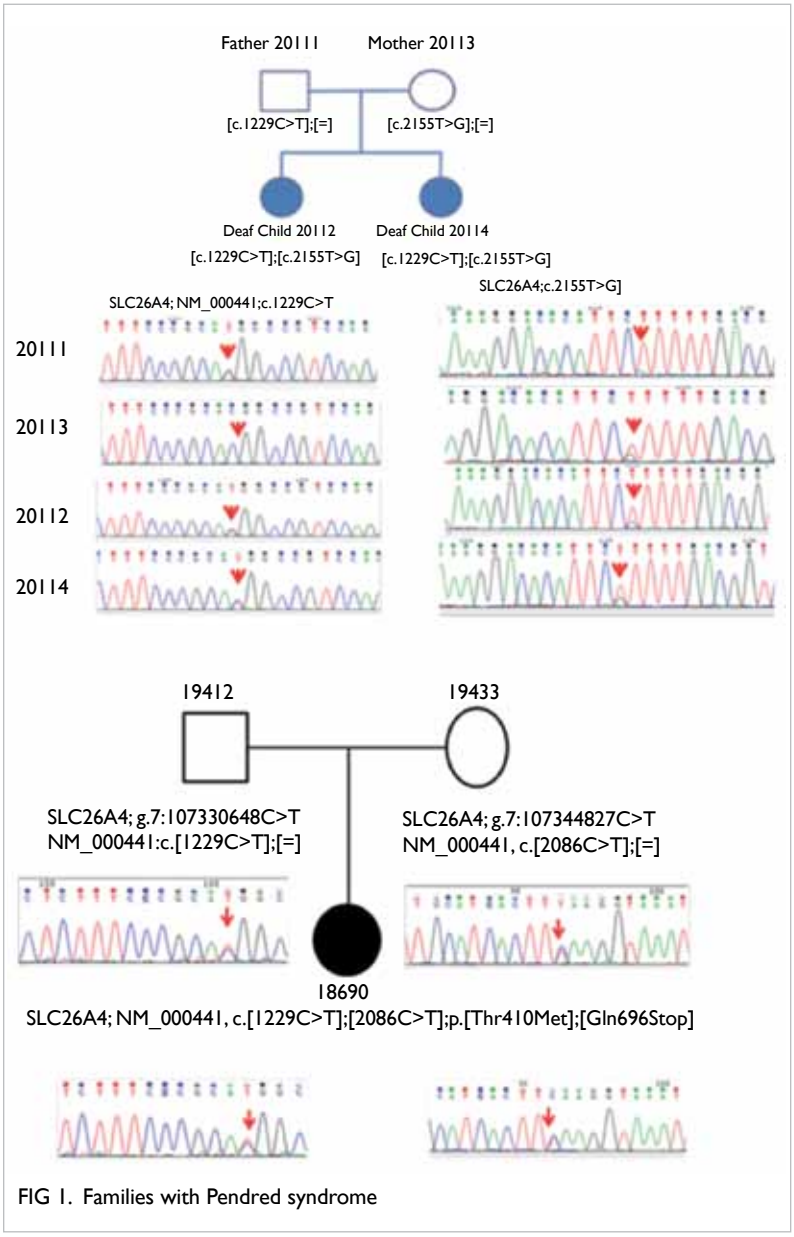
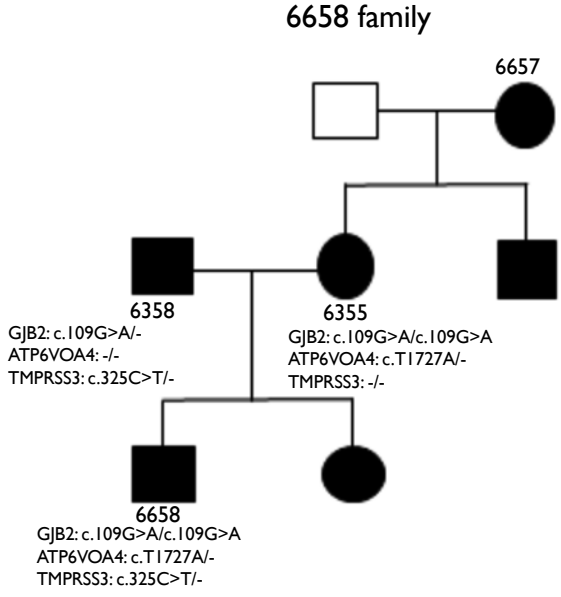


FIG 1. Families with Pendred syndrome

Discussion

Our study indicates a high carrier frequency of *GJB2* c.109G>A in a Chinese population. The total heterozygous and homozygous carrier frequencies of *GJB2* c.109G>A were as high as 10.29% and 0.22%, respectively; the heterozygous frequency of this mutant is comparable to the carrier frequency of 11.6% in a Taiwanese population. This finding indicates that *GJB2* c.109G>A is common in Asians



ATP6VOA4[NM_020632]: c.T1727A/, p.F576Y, Chr7:138417803(hg19), no report b4, in silico predicted as pathogenic
TMPRSS3: [NM_024022]: DFNBB/10, c.325C>T, p.Arg109Trp, Clinvar reported as pathogenic

FIG 2. A family with a neonate with severe hearing loss

but rare in Caucasians. Nonetheless, newborns who carry this homozygous mutation showed variable phenotypes, with a high OAE pass rate (9/13) in our cohort. This *GJB2* c.109G>A genotype was first reported as a polymorphism and has been suggested to be pathogenic and a cause of mild hearing loss. In our cohort, the carrier frequency of *GJB2* c.109G>A and *SLC26A4* c.1174A>T and c.2168A>G differed considerably from the global minor allele frequency calculated from the 1000 Genomes dataset. This difference suggests that the comparison of mutation frequency should be done among ethnically or geographically matched populations.

The use of the OAE test for early diagnosis is well established in Hong Kong. Considering its false-positive rate of 2.5% to 8%, follow-up auditory examinations to confirm the diagnosis are warranted. Our established test offers a genetic diagnosis and provides better sensitivity and specificity for managing hearing-loss patients and genetic counselling. Genetic diagnosis helps identify potential late-onset hearing loss and facilitate long-term follow-up of all pre-symptomatic cases.

Acknowledgements

This work was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120256). We thank our clinical colleagues who helped to recruit cases, patients who participated in the study, and colleagues for helping ensure a smooth delivery of this project.

Ethical Approval

This study was approved by the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee (CRE-2011.098).

Declaration

The authors have no conflicts of interest to disclose.

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Molecular diagnosis of severe combined immunodeficiency using whole-exome sequencing

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KEY MESSAGES

1. We studied 50 cases of severe combined immunodeficiency from 33 families using a combination of genetic and genomic approaches.
2. Molecular diagnosis was successfully made in 19 of the 33 families.
3. A novel primary immunodeficiency disease gene—*RASGRP1*—was identified.
4. In a number of cases, we gained new understanding of genotype-phenotype correlations, such as mutations in *TTC7A* and in desmoplakin.
5. We gained valuable experience in making a molecular diagnosis of severe combined immunodeficiency; such diagnosis may help determine the causal mutations in this group of patients.

Hong Kong Med J 2018;24(Suppl 3):S15-7
HMRF project number: 01120846

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This summary is partly based on studies first reported in: (1) Yang W, Lee PP, Thong MK, et al. Compound heterozygous mutations in *TTC7A* cause familial multiple intestinal atresias and severe combined immunodeficiency. Clin Genet 2015;88:542-9. (2) Mao H, Yang W, Latour S, et al. *RASGRP1* mutation in autoimmune lymphoproliferative syndrome-like disease. J Allergy Clin Immunol 2017. pii: S0091-6749(17)31756-6 [Epub ahead of print].

Introduction

This study aimed to provide a molecular diagnosis for patients with severe combined immunodeficiency (SCID)—a severe form of primary immunodeficiency diseases (PID)—who were negative in candidate gene screening, by using a combination of genetic and genomic approaches. This study also aimed to discover new genes or signal transduction pathways involved in SCID, to survey the spectrum of causal mutations for SCID in a Chinese population, and to develop algorithms and bioinformatics pipelines/tools for whole-exome sequencing (WES) data analysis that can be applied to the clinical diagnosis of rare Mendelian diseases. We successfully conducted WES on the study patients and identified novel SCID genes. Our approach facilitates an understanding of the disease and enables the provision of definitive genetic counselling for affected families. We gained valuable experience in WES data analysis for the diagnosis of monogenic diseases. We have also trained graduate students in the analysis of relevant genetic and genomic data.

Methods

We performed WES on genomic DNA extracted from peripheral blood leukocytes of 50 cases of severe combined immunodeficiency from 33 families. Coding exons of almost 20 000 genes in the human genome were enriched before being sequenced using next-generation sequencing technology, mainly with the HiSeq 2500 System (Illumina, San Diego [CA], United States) on 2x150-bp paired reads. The effect of the genetic variants detected on the encoded proteins was analysed, as well as their population frequency based on data from public and proprietary domains, the function of the genes, and the assumed genetic inheritance mode in the affected families. Potential causal mutations were confirmed by Sanger sequencing, and their frequency in the local population was examined by Sequenom Massarray, a MALDI-TOF mass spectrometry–based genomics analytical method (San Diego [CA], United States). For a number of samples, RNA-seq (RNA sequencing) was used to aid the identification of causal mutations by detecting crucial gene expression changes and alternative splicing aberrations. Owing to the inadequacy of analysis tools for next-generation sequencing platform data, we developed an in-house tool and a genetic variant database that was more relevant to the local population. For exome sequencing data analysis, we have an established pipeline in the laboratory.

For RNA-seq data analysis, quality control with the FastQC tool (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) for raw reads included checks of sequence quality, GC content, overrepresented *k*-mers, and duplicated reads in order to detect sequencing errors, polymerase chain reaction (PCR) artefacts, or contaminations. Reads were mapped to a reference genome with a gapped mapper STAR.[1] Quantification was primarily based

on the number of reads that mapped to each transcript sequence using HTSeq-count.[2] As we did not have exactly matched controls, RNA-seq data from a number of samples from NCBI Gene Expression Ominibus were used as controls. Those samples were derived from peripheral blood mononuclear cells, the same cell source as our patient RNA-seq data. Differential expression analysis was performed using DESeq2,[3] which takes raw read counts as input and introduces possible bias sources into the statistical model to perform an integrated normalisation as well as a differential expression analysis.

The functional analyses of the RNA-seq data were divided into three major components: expression level analysis to detect deletion of a candidate gene or disturbance of a major signal transduction pathway; variant calls to detect mutations that might be missed by WES; and detection of aberrant splicing variant or absence of major functional transcripts. Studying differentially expressed genes allowed the detection of complete loss of expression of a gene or a major transcript for a gene. Complete loss could indicate a homozygous deletion of a locus that might not be reflected by WES data, as variations in coverage depth are intrinsic to exome sequencing data. Furthermore, upregulation or downregulation of a group of genes might indicate aberration of a major signalling transduction pathway. Thus, the differentially expressed genes were assessed using pathway analysis tools such as ToppGene (https://toppgene.cchmc.org/) and David (https://david.ncifcrf.gov/). The variants identified from RNA-seq data were systematically compared with those from the WES data to try to detect variants that might be missed. Different transcripts from alternative splicing for a given gene were evaluated to detect genes with potential mutations that could affect splicing, thereby resulting in either the expression of an aberrant splicing transcript or the absence of a major functional transcript. For the latter, we focused on genes without detection of a major constitutive coding exon(s) or genes that were missing the established major transcript.

Results and Discussion

In this study, we discovered a novel PID gene, *RASGRP1*, which will aid future efforts in diagnosis for this group of patients.[4] In addition, we identified a de novo mutation in desmoplakin, and identified causal mutations in *TTC7A* in a patient with immunodeficiency and intestinal atresias.[5] We identified known causal genes in 19 cases among 33 PID families, thus providing a molecular diagnosis for these families. Furthermore, we developed a novel tool for WES analysis that specifically focuses on SCID, and we provided training to students in genomic data analysis during this process.

Using WES in combination with in-depth analysis and other genetic and genomic approaches, we identified novel genes for SCID and also made a molecular diagnosis in 19 affected patients. These novel findings add to our understanding of SCID and will help our future work in molecular diagnosis for this group of diseases. This approach is important to enable individualised treatment of the disease and for developing new intervention paradigms and new drug targets for SCID. Although identifying genes known to cause SCID is not itself novel, the findings inform us about the mutation spectrum in the local population for this group of diseases—a vital piece of information that will help future genetic screening. Definitive molecular diagnosis will ensure accurate genetic counselling and provide valuable information for prenatal diagnosis if needed. There were a number of cases in which the molecular diagnosis prompted us to re-examine the patient's clinical phenotype. This has enabled a new understanding of genotype-phenotype correlations.

There were a number of families in whom no causal mutations could be identified, even after applying state-of-the-art technologies including WES and RNA-seq. This finding demonstrates the limitations of the technology and the lack of an in-depth understanding of relevant genes and disease mechanisms. Nonetheless, we have been able to build on our experience of next-generation sequencing analysis for molecular diagnosis and to train graduate students to handle data analysis and to make a diagnosis.

From this study and related work, we conclude that WES is a cost-effective approach for molecular diagnosis of PID and other monogenic diseases. A number of candidate genes have usually been screened prior to application of WES. Nonetheless, owing to the enormous heterogeneity of SCID, candidate gene screening is inefficient and time-consuming. With its power and ever-decreasing cost, WES is becoming a mainstream cost-effective approach to the molecular diagnosis of SCID. Among our cases was a case of radiation-sensitive SCID, and mutations in *DCLRE1C* were correctly suspected. Despite this finding, genetic screening using PCR and Sanger sequencing was negative. Yet, homozygous deletion of multiple exons of *DCLRE1C* and adjacent genes was detected by WES. We speculate that the reason for the lack of detection by Sanger sequencing was because of trace contamination of normal DNA that was enormously amplified by the 40-plus cycles of PCR. The issue of trace contamination is well tolerated by next-generation sequencing technology, because only about 10 cycles of PCR amplification were applied during library construction.

Acknowledgements

This study was supported by the Health and Medical

Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120846). We thank the patients and the families for participating in this research.

Ethical Approval

This study was approved by Institutional Research Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster (UW12-211). Informed consent was obtained from each participant.

Declaration

The authors have no conflicts of interest to disclose.

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Apathy after stroke: potential risk factors and magnetic resonance imaging markers

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KEY MESSAGES

1. The prevalence of post-stroke apathy (PSA) at 3 months was 24.7% among 267 stroke survivors.
2. Risk factors associated with PSA were older age, male sex, history of hyperlipidaemia, depressive symptoms, a lower level of cognitive function, and functional disability. A pontine acute infarct on magnetic resonance images was an independent predictor of PSA at 3 months.
3. PSA persisted in 51.1% of 47 stroke patients at 9 months and 41.7% of 12 patients at 15 months.
4. The onset of PSA can be delayed. Among 201 non-PSA patients at 3 months, 21 developed PSA at the later stage of rehabilitation (9 or 15 months).
5. The psychological burden of PSA should not be neglected. Early identification and treatment are essential.

Hong Kong Med J 2018;24(Suppl 3):S18-20
HMRF project number: 01120376

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This summary is based on studies first reported in: (1) Tang WK, Lau CG, Mok V, Ungvari GS, Wong KS. Apathy and health-related quality of life in stroke. Arch Phys Med Rehabil 2014;95:857-61. (2) Tang WK, Caeiro L, Lau CG, Mok V, Ungvari GS, Wong KS. Apathy and suicide-related ideation 3 months after stroke: a cross-sectional study. BMC Neurol 2015;159:60.

Introduction

Apathy is defined as a decrease in goal-directed behaviour attributable to loss of motivation. It is characterised by a general lack of emotion, interest, or concern. Stroke survivors with apathy are commonly described as having lost interest, being unmotivated, being unable to get going, or being "content to just sit there".[1] Post-stroke apathy (PSA) is a debilitating condition, and its prevalence is 20% to 25% in stroke patients.[2] The possible clinical correlates of PSA include older age, a low educational level, depression, low cognitive impairment, and poor physical functioning.[3] However, PSA is often undiagnosed and thus untreated, even though it may impair stroke recovery.[3] The clinical course of PSA has not been extensively studied, and there have been only a few structural brain imaging studies on PSA. Some researchers have reported an association between PSA and infarcts in the posterior limb of the internal capsule, basal ganglia, and white matter hyperintensities, whereas others have reported no association between lesion location and PSA. Past studies also have limitations, including small sample size, inclusion of patients with psychiatric diseases,[4] a lack of standardised PSA assessments,[3] and an absence of detailed radiological examination.[3,4] This study aimed to evaluate the clinical and magnetic resonance imaging (MRI) correlates of PSA in a cohort of stroke survivors, and to describe the 12-month course of PSA.

Methods

A total of 1201 patients with first-ever or recurrent acute ischaemic stroke were admitted to the Acute Stroke Unit of the Prince of Wales Hospital between April 2014 and April 2016. Of these patients, 656 underwent MRI, and 267 of these fulfilled the inclusion criteria. The inclusion criteria were (1) Chinese ethnicity, (2) Cantonese as the primary language, (3) age of at least 18 years, (4) well-documented first or recurrent acute ischaemic stroke within 7 days of admission, and (5) ability to provide informed consent. The exclusion criteria were (1) transient ischaemic attack, cerebral haemorrhage, subdural haematoma, or subarachnoid haemorrhage; (2) a history of a central nervous system disease such as tumour, Parkinson's disease, or dementia; (3) a history of depression or other psychiatric disorder; (4) a Mini-Mental State Examination (MMSE) score of <20; (5) severe aphasia or auditory or visual impairment; (6) physical frailty; (7) recurrence of stroke before the 3-month assessment; and (8) contraindications to MRI such as a pacemaker in situ.

A research nurse collected patients' demographic and clinical data and assessed stroke severity within 2 days of admission, using the National Institutes of Health Stroke Scale. A research assistant administered the MMSE, Barthel Index assessment, and 15-item Geriatric Depression Scale (GDS) at three timepoints: 3, 9, and 15 months

after onset of the index stroke. Three months after the onset of the index stroke, a psychiatrist who was blinded to the radiological data administered the clinician's version of the 18-item Apathy Evaluation Scale (AES-C), using a 4-point Likert scale in which higher scores indicated more severe apathy. The AES-C has good reliability and validity and has been used to measure PSA, which is defined as an AES-C score of ≥37.

Diffusion-weighted and conventional MRI was performed with a 1.5-T system (Sonata; Siemens Medical, Erlangen, Germany) within 7 days of admission. The number and volume of acute infarcts in different structures, number and location of cerebral microbleeds, and Fazekas score for extent of white-matter hyperintensities were assessed by a neurologist who was blinded to the PSA diagnosis.

The demographic, clinical, and radiological variables of the PSA group were compared with those of the non-PSA group using the chi-square test, Student's *t* test, or Mann-Whitney *U* test, as appropriate. Multivariable regression was performed to determine risk factors of PSA. If the correlation coefficient between two variables was ≥0.50, then only one of them was entered into the regression model to avoid co-linearity.

Results

The 267 recruited patients (108 women and 159 men) had a mean ± standard deviation age of 66.4±10.7 years, duration of education of 7.0±4.4 years, and National Institutes of Health Stroke Scale score on admission of 3.1±3.9.

At 3 months, 66 (24.7%) patients were diagnosed with PSA. Independent predictors of PSA at 3 months were the number of acute infarcts on MRI scans (odds ratio [OR]=1.226, 95% confidence interval [CI]=1.004-1.497; P=0.046), presence of pontine acute infarcts on MRI scans (OR=2.666, 95% CI=1.021-6.961; P=0.045), MMSE score (OR=0.850, 95% CI=0.747-0.967; P=0.014), and functional disability measured by the Barthel Index (OR=0.714, 95% CI=0.557-0.914; P=0.008).

At 9 months, 67 (37.9%) of 177 patients examined were diagnosed with PSA. A higher degree of cognitive function (OR=0.803, 95% CI=0.690-00.934; P=0.005) was a protective factor of PSA. Independent risk factors of PSA were male sex (OR=2.530, 95% CI=0.135-5.637; P=0.023), older age (OR=1.049, 95% CI=1.006-1.088; P=0.023), a history of hyperlipidaemia (OR=2.418, 95% CI=1.126-5.192; P=0.024), and a history of depression defined as a GDS score of ≥7 (OR=11.416, 95% CI=2.535-51.406; P=0.002).

At 15 months, 67 (39.4 %) of 170 patients examined were diagnosed with PSA. The regression model indicated that independent predictors of PSA were GDS score (OR=1.370, 95% CI=1.177-1.593; P<0.001) and MMSE score (OR=0.851, 95% CI=0.764-0.946; P=0.003).

Regarding the clinical course of PSA of recruited patients, 66 were diagnosed with PSA at 3 months, of whom 47 attended the 9-month follow-up visit, and 24 (51.1%) of these were diagnosed as still having PSA. Of 12 patients who attended the 15-month follow-up visit, 5 (41.7%) were diagnosed as still having PSA. Of the 201 patients with no PSA at 3 months, 130 attended the 9-month follow-up visit, of whom 43 (28.7%) were diagnosed with PSA. Of 67 patients who attended the 15-month follow-up visit, 21 (31.3%) were diagnosed with PSA.

Discussion

To the best of our knowledge, this is the first structural MRI study to determine association between pontine infarcts and the risk of PSA. Independent risk factors of PSA were older age, male sex, a history of hyperlipidaemia and depressive symptoms at 3 months post-stroke, cognitive and physical function at 3 months post-stroke, and pontine infarcts. Post-stroke apathy can have a late onset and run a chronic course. Older age and male sex were associated with PSA at the 9-month follow-up visit. A meta-analysis has reported that patients with PSA are 3.8 years older, on average, than those without.[2] The greater burden of vascular lesions borne by elderly patients has been proposed as a potential cause of the increased likelihood of PSA. Although male sex was a predictor of PSA at the 9-month follow-up visit, it was of only borderline significance in the univariate analysis.

Post-stroke apathy was associated with depressive symptoms and a lower level of cognitive and physical function at 3, 9, and 15 months post-stroke. In a meta-analysis, depression was more common and depressive symptoms were more severe in those with PSA.[2] The association of PSA with cognitive impairment may be explained by advanced age and the underlying brain damage that results from stroke. Functional disability has also been reported to be associated with PSA.

The presence of pontine acute infarcts was predictive of PSA at 3 months. This finding is consistent with one study that reported more apathy in 16 patients with subtentorial stroke than in patients with parietal-occipital lobe infarcts. Structural brainstem abnormalities or dysfunction have been demonstrated in apathy in progressive supranuclear palsy.

In our study, about 40% of PSA persisted up to the 15-month follow-up appointment, suggesting chronicity. The prevalence of new-onset PSA was 21.3% at 9 months and 24.1% at 15 months.

In a cross-sectional study, the Neuropsychiatric Inventory Apathy score was significantly higher in stroke patients than in normal controls at 6 months and 1 year, rather than at 3 months.[5] In addition, delayed onset has been observed in other post-stroke psychiatric comorbidities. Underlying accumulative vascular lesions are the main cause of increasing apathy in later stages of stroke rehabilitation.

Limitations

The main limitation of this study is potential selection bias, as only a relatively small proportion of the original cohort was examined, possibly limiting the generalisability of the findings. The drop-out group had more severe stroke. Because patients with previous stroke were recruited, pre-existing infarcts may have contributed to PSA development.

Acknowledgement

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120376).

Ethical Approval

This study was approved by Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee.

Declaration

The authors have no conflicts of interest to disclose.

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Ictal intracranial electroencephalography using wavelet analysis of high-frequency oscillations in Chinese patients with refractory epilepsy

HW Leung*, WS Poon, PKL Kwan, CHT Lui, TL Poon, ELY Chan, SC Yuen

KEY MESSAGES

1. Both intracranial electroencephalography and detection of wavelet-transformed high-frequency oscillations (HFOs) may be beneficial in surgery for patients with focal epilepsy.
2. Detection of wavelet-transformed HFOs may increase the percentage of patients eligible for resective surgery by 5% to 6.5%, compared with intracranial electroencephalography alone.
3. Detection of wavelet HFOs may improve surgical outcome by 17% to 18%, compared with intracranial electroencephalography alone, and by 30% if no intracranial electroencephalography is used.
4. High-frequency oscillations that are detected at the onset of seizure help determine seizure outcome.
5. Cortical areas that demonstrate hyperexcitability may be associated with HFOs.

Hong Kong Med J 2018;24(Suppl 3):S21-3
HMRF project number: 01120726

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This summary is based on the study first published in: Leung H, Zhu CX, Chan DT, et al. Ictal high-frequency oscillations and hyperexcitability in refractory epilepsy. Clin Neurophysiol 2015;126:2049-57.

Introduction

Epilepsy is a neurological disease that places a heavy burden on society. Approximately 68 000 patients in Hong Kong have some form of seizure disorder,[1] and one-third of these patients have seizures that are refractory to medical treatment. Surgery may provide a cure in due course, but identification of seizure focus is necessary for success. In patients with a clear and resectable structural lesion, surgery may proceed after video electroencephalography, magnetic resonance imaging, and clinical psychological testing. Nonetheless, some patients do not appear to have a resectable lesion according to these methods.

High-frequency oscillations (HFOs) refer to electrographic activity of 80 to 500 Hz. It is hypothesised that HFOs can be biomarkers for epilepsy.[2] Wavelet transformation may accurately depict HFOs. We propose that detection of wavelet-transformed HFOs of a seizure may help determine the seizure-onset zone that is essential for epilepsy surgery.

Methods

A total of 128 patients with refractory epilepsy underwent resective surgery in our hospitals between July 2013 and June 2015. Of these patients, 15 women and 19 men (mean age, 34 years) gave informed consent to undergo intracranial electroencephalography. These patients underwent implantation of grid or strip electrodes with a range of configurations to delineate seizure foci. Episodes of stereotypic seizure with clinical manifestations were recorded in 3-minute epochs, adopting bipolar montage and good technical quality. Each epoch covered the entire seizure and centred on a quarter of its own length from the onset of seizure. Electroencephalographic findings for each seizure were first analysed visually by a neurologist, followed by off-line export of data for wavelet analysis. The number of scales that corresponded to the range of 80 to 500 Hz was used. The algorithm was generated using a MATLAB platform. The mother wavelet used was biorthogonal 6.8. The density of HFOs was calculated by a peak-to-trough power ratio of 50 to 70. If the ratio fell below 10, the electrode position might not have represented the seizure onset zone.

A previous cohort served as a pilot control group in which the percentage of patients eligible for resective surgery was 70% and the rate of good surgical outcome was 57%.[3] During the recording procedures, conventional frequency ictal patterns,

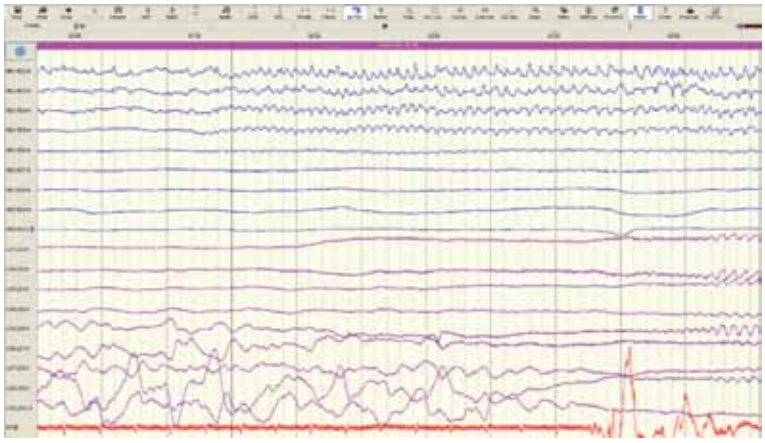

FIG 1. Electrographic record of a difficult-to-locate onset that may be amenable to testing for high-frequency oscillations

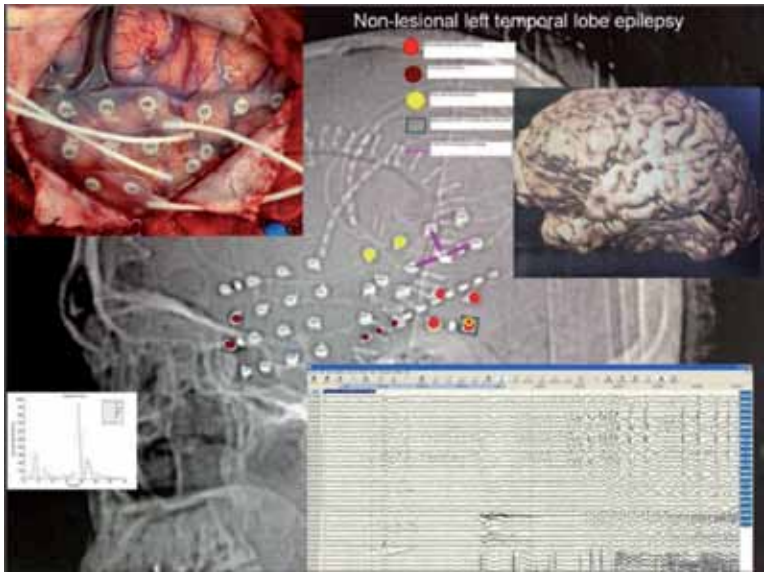

FIG 2. Sampling electrodes placed over a curvature of neocortical area and high-frequency oscillations

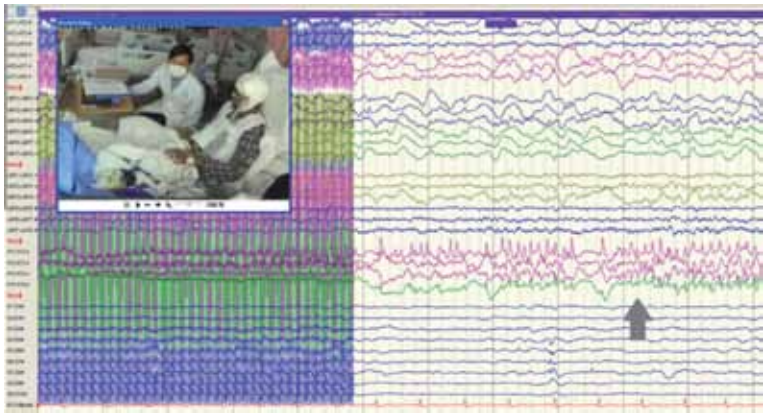

FIG 3. Conditions of hyperexcitability

hyperexcitability, and radiological lesion were also recorded.

An ancillary study included the technical aspect of electroencephalographic data. Hyperexcitability was defined as the appearance of after-discharges or clinical seizures following electrical stimulation (50 Hz, biphasic, pulse width of 0.5 ms, 5 s, 5 mA). The mean proportion of HFOs among resected channels was compared with that of the conventional frequency ictal pattern, hyperexcitability, and radiological lesion.

Results and Discussion

The proportion of patients who were eligible for resective surgery was 76.5% following review of wavelet-transformed HFOs and 75% following visual analysis of HFOs. Thus, an increase of 5% to 6.5% was expected when wavelet-transformed HFOs were analysed. The proportion of patients who attained good surgical outcome with accurate identification of seizure-onset zone was 71.4% following review of wavelet-transformed HFOs, compared with 75% following visual analysis of HFOs. This represented an increase of 17% to 18% when compared with no visual analysis of HFOs, and an increase of 30% when compared with no intracranial electroencephalography.

By testing for HFOs, we demonstrated a safe and fast methodology to determine the laterality of onset for patients with bilateral mesial temporal scleorsis (Fig 1). In patients in whom electrographic signals were sampled in the greater curvature of a neocortical surface, the number of channels involved initially may have been so extensive that rapid identification of foci was not feasible. Our mathematical representation identified the distinctive region with HFOs and added strength to information not visible to the naked eye (Fig 2). In addition, our analysis showed additional evolution of discharges throughout the seizure epoch that was not uniform. Fast activity was evident at the very first moment of the seizure, followed by a decrease in power towards the mid-portion. As the seizure epoch concluded, spectral power was regained, culminating in final, abrupt cessation of seizure. This phenomenon was not observable by visual analysis of HFOs. Hyperexcitability co-occurred with HFOs, conventional frequency ictal patterns, and radiological lesions (Fig 3).

Combining two or more modalities may improve selection of candidates for surgery. Our data suggest that when both wavelet-transformed HFOs and hyperexcitability are used, sensitivity can be maintained at 100% (95% confidence interval [CI]=0.52-1) and specificity may be increased from 66.7% (95% CI=0.31-0.91) to 75% (95% CI=0.36-0.96), compared with wavelet-transformed HFOs alone.

Our study demonstrated that by testing for wavelet-transformed HFOs, patients who would otherwise be denied surgery may receive a potential cure. Our study has used an effective research platform in electroencephalography whose results concur with those of other studies.[4,5] We are interested in ictal HFOs because they have been revealed during intracranial recordings. Identifying the moment of seizure provides researchers with the strongest evidence of localisation and lateralisation. Surgery improves seizure outcome and is feasible in refractory epilepsy, and patient quality of life can be improved. This project may improve public awareness and institutional priority and in turn, benefit patients with epilepsy.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120726). We thank Ms Kwun-Lin Man and Ms Roxanna Liu for their coordination of the project, and Dr XL Zhu and Prof KS Wong for their support in all aspects of clinical research.

Ethical Approval

Informed consent was obtained from each participant.

Declaration

The authors have no conflicts of interest to disclose.

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Early neurodegenerative biomarkers and clinical outcome in psychiatric patients with rapid eye movement sleep behaviour disorder: a prospective study

YK Wing*, SP Lam, VCT Mok, J Zhang, JMY Tsoh, EYL Leung

KEY MESSAGES

1. Patients who have rapid eye movement sleep behaviour disorder (RBD) as well as psychiatric illness (pRBD) may have a neurodegenerative outcome. The annual incidence of RBD was 1.9% in psychiatric patients. The incidence of Parkinson's disease in pRBD patients was 1.1%.
2. In most patients, RBD runs a persistent course. Despite symptomatic drug treatment, RBD symptoms and consequent sleep-related injury are still common.
3. Compared with psychiatric patients without comorbid RBD, pRBD patients have more prominent symptoms of depression and anxiety.
4. Persistent olfactory dysfunction is a feature in pRBD.

Hong Kong Med J 2018;24(Suppl 3):S24-8
HMRF project number: 01120326

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This summary is based on a study first published in: Wing YK, Lam SP, Zhang J, et al. Reduced striatal dopamine transmission in REM sleep behavior disorder comorbid with depression. Neurology 2015;84:516-22.

Introduction

Rapid eye movement (REM) sleep behaviour disorder (RBD) is a novel and distinct parasomnia characterised by recurrent dream-enacting behaviours and polysomnographic features of a loss of normal REM sleep–related muscle atonia. In older patients, RBD (idiopathic RBD) is a precursor to neurodegeneration. Some patients with RBD who also have psychiatric illness (pRBD) show comparable clinical symptoms and abnormal REM-related electromyographic (EMG) muscle activities to idiopathic RBD patients.[1-3] The manifestation of RBD in these patients is increasingly recognised as an entity beyond a drug-induced condition. In addition, the REM-related EMG activities in pRBD patients are related to the severity of the mental illness. Preliminary results suggested that pRBD patients had more olfactory and dopamine dysfunction.[4,5] This study aimed to establish the longitudinal course and outcome of pRBD in terms of clinical symptoms, polysomnographic features, neurocognitive profile, and dopamine neurotransmission.

Methods

This was a prospective follow-up study of a cohort (including cases and controls) established in our previous cross-sectional case-control study in 2008.[1] It included a comprehensive phase 1 assessment of pRBD cases and two control arms that comprised psychiatric patients with no RBD symptoms (pControl) and healthy controls (hControl) to establish the longitudinal course, outcome, and neurocognitive profile of the participants. A subset of participants were recruited for phase 2, in which a neuroimaging study was conducted to identify any potential dopamine dysfunction.

We aimed to (1) establish the longitudinal course of RBD in terms of polysomnographic abnormalities of persistence and progressive increase in REM-related EMG activities, (2) compare the neurocognitive profile and any changes over time between case and control groups, and (3) explore the potential signs of early neurodegeneration (in terms of dopaminergic transmission abnormality) using neuroimaging. We hypothesised that: (1) RBD is a sustained and progressive condition in pRBD patients, and REM-related EMG activities will increase with time in these patients; (2) the neurocognitive profile would show a more prominent decline in the pRBD group over time compared with the control groups, particularly olfactory function and colour vision; and (3) pRBD patients would show early dopaminergic transmission abnormalities as demonstrated on neuroimaging.

Primary outcomes included the magnitude of

REM-related EMG activities and change in olfactory and visual neurocognitive profile, as measured by the olfactory identification test and Farnsworth-Munsell-100 Hue test. Secondary outcomes included neurocognitive performance (as measured by Mattis Dementia Rating Scale, Hong Kong List Learning test, Rey-Osterrieth complex figure, and trial-making test), dopamine transmission (quantified by neuroimaging), and incidence of new-onset clinical neurodegenerative diseases.

Data were analysed by SPSS (Windows version 22.0; IBM Corp, Armonk [NY], United States). Repeated measures analysis of variance by a general linear model was used for continuous data with a normal distribution. Skewed continuous data were log-transformed or square root–transformed for further general linear model analysis. For categorical data, a generalised linear model was used for repeated measurement. For variables in which only follow-up data were available (eg neurocognitive tests), analysis of variance or the Kruskal-Wallis test was used for three-group comparison, followed by post-hoc analysis, as appropriate.

Results

A total of 177 participants were recruited at baseline (Fig). One pRBD patient died and three healthy controls were excluded owing to subsequent development of mental illness (n=2) or pregnancy (n=1). Among 173 participants, 120 (69.4%) completed the follow-up study (phase 1) and comprised 39 pRBD patients, 38 pControls, and 43 hControls.

Longitudinal outcomes

At follow-up, of 39 pRBD patients, two men developed Parkinson's disease (PD) and had RBD symptoms at the age of 55 years. The overall prevalence and incidence of developing PD in pRBD patients were 5.1% and 1.1%, respectively. In 37 participants who remained free of any neurodegenerative disorder, 31 (83.8%) reported persistent RBD symptoms (defined as a RBD Questionnaire total score of ≥22 and a RBD Questionnaire factor 2 score of ≥8), and six (16.2%) reported no active RBD symptoms over the past year (five were female), of whom three were prescribed symptomatic drug treatment for RBD, namely clonazepam and melatonin. Regarding their antidepressant use at follow-up, two had stopped antidepressant use, two had switched the antidepressant from a selective serotonin reuptake inhibitor to a dopamine non-adrenaline reuptake inhibitor, and the remaining two had not altered their antidepressant regimen. For those with persistent RBD symptoms, 41.9% reported a history of sleep-related injury to themselves or bed-partners in the past year and 58.1% were taking medications for RBD symptoms.

For the pControl group, three participants developed RBD: two were taking the same antidepressant regimen at baseline and one had augmented treatment with two different classes of antidepressants. None was prescribed any medication for RBD symptoms. The prevalence and incidence of developing RBD in psychiatric patients were 7.9% and 1.9%, respectively. For the hControl group, there was no new incidence of RBD or neurodegenerative diseases.

Clinical and demographic characteristics

To compare the clinical characteristics of psychiatric patients with or without RBD, only patients without any change to their RBD status or neurodegenerative disease were included. Patients who developed PD in the pRBD group, and those who developed RBD in the pControl group were excluded from analysis.

There were no significant differences in sex or age at follow-up across the three groups (Table). The mean duration of follow-up was slightly longer in the pRBD group than the hControl group. Most (>90%) participants in the pRBD and pControl groups were diagnosed with major depressive disorder (MDD). There were five pRBD patients who had co-morbid

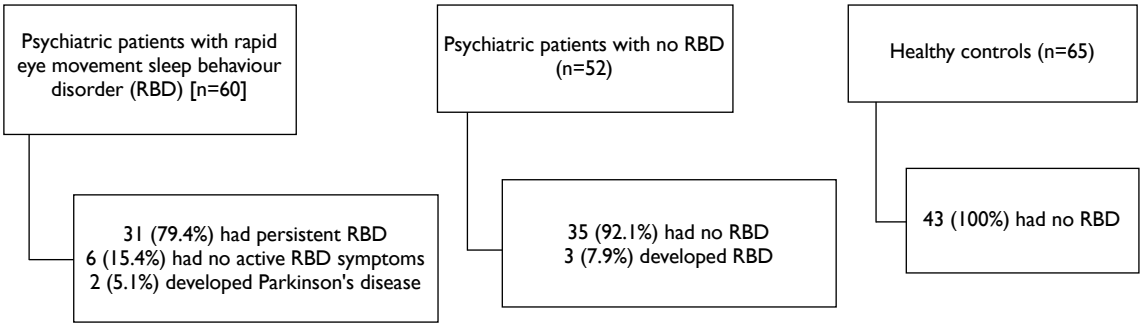

FIG. Flowchart showing longitudinal follow-up

MDD and post-traumatic stress disorder. Both pRBD and pControl groups had comparable psychotropic use, although the pRBD group had a higher use of mood stabilisers (32.3% vs 8.6%; P<0.05) and clonazepam (51.6% vs 5.7%; P<0.01).

Regarding clinical symptoms, the pRBD group had a significantly higher RBD questionnaire score than the other two groups at both baseline and follow-up (F(2,105)=122.8; P<0.01). The pRBD group also had a higher Hospital Anxiety and Depression Scale score (F(2,99)=27.386; P<0.01) and Beck Depression Inventory score (F(2,95)=22.427; P<0.01) than the other two groups at both baseline and follow-up. None of these variables showed any significant difference during the follow-up period. The Unified Parkinson's Disease Rating Scale motor score of pRBD patients at both baseline and follow-up was higher than that of the other two groups (F(2,77)=7.186; P<0.01); there was a significant change in the score during the follow-up period and there was a significant interaction between time and groups (F(2,77)=3.227; P<0.05). In particular, the pRBD group had a higher magnitude of increase in the score.

For total REM-related EMG activities, there was a significant difference during the follow-up period (F(1,99)=40.063; P<0.01) and the pRBD group had a significantly higher REM-related EMG score at both baseline and follow-up (F(2,99)=16.088; P<0.01) than the other two groups. Similar significant trends

TABLE. Clinical characteristics of study participants

Variable	Psychiatric patients with RBD (n=31)*	Psychiatric patients without RBD (n=35)*	Healthy controls (n=43)*	P value
Male	16 (51.6)	9 (25.7)	23 (53.5)	<0.05
Age at follow-up, y	49.8±10.4	52.7±11.0	50.1±7.3	NS
Education				<0.05
Illiterate/primary	4 (12.9)	9 (25.7)	2 (4.7)	
Secondary or above	27 (87.1)	26 (74.3)	41 (95.3)	
Follow-up duration, m	56.4±14.6	52.5±14.5	48.2±16.3	NS
Psychiatric diagnosis at baseline				
Major depressive disorder	29 (93.5)	33 (94.3)	-	NS
Bipolar affective disorder	2 (6.5)	1 (2.9)	-	NS
Post-traumatic stress disorder	5 (16.1)	0	-	<0.05
Schizophrenia/psychosis	0	1 (2.9)	-	NS
Psychiatric diagnosis duration, m	139.8±68.1	133.0±73.1	-	NS
Psychotropics at baseline				
Antidepressants	29 (93.5)	33 (94.3)	-	NS
Antipsychotics	6 (19.4)	7 (20.0)	-	NS
Mood stabiliser	6 (19.4)	3 (8.6)	-	NS
Benzodiazepines	17 (54.8)	5 (14.3)	-	<0.01
Non-benzodiazepine hypnotics	5 (16.1)	8 (22.9)	-	NS
Clonazepam	11 (35.5)	2 (5.7)	-	<0.01
Melatonin	1 (3.2)	1 (2.9)	-	NS
Psychotropics at follow-up				
Antidepressants	26 (83.9)	29 (82.9)	-	NS
Antipsychotics	9 (29.0)	12 (34.3)	-	NS
Mood stabiliser	10 (32.3)	3 (8.6)	-	<0.05
Benzodiazepine	17 (54.8)	4 (11.4)	-	<0.01
Non-benzodiazepine hypnotics	4 (12.9)	4 (11.4)	-	NS
Clonazepam	16 (51.6)	2 (5.7)	-	<0.01
Melatonin	1 (3.2)	1 (2.9)	-	NS
Prazosin	6 (19.4)	0	-	<0.01

Abbreviations: NS = not significant; RBD = rapid eye movement sleep behaviour disorder

* Data are presented as mean ± standard deviation or No. (%) of participants

were also found in tonic and phasic activities. There was no interaction between time and groups.

Neurocognitive markers

The pRBD group had more olfactory dysfunction at baseline than the pControl and hControl groups (41.9% vs 14.7% vs 16.3%; P<0.05). At follow-up, the prevalence of olfactory dysfunction remained higher in the pRBD group but not significantly (27.6% vs 17.1% vs 11.6%; P=0.22). More pRBD patients had persistent olfactory dysfunction at both baseline and follow-up (24.1% vs 5.9% vs 4.7%; P<0.05). Regarding colour vision, all three groups showed significant changes with time but there was no difference across the three groups.

For trial-making test, pRBD and pControl groups had significantly poorer performance than the hControl group (93.2±44.4 vs 108.7±51.7 vs 69.8±19.6; P<0.01), but the two psychiatric groups were comparable (post-hoc analysis: pRBD > hControl; P<0.05 and pControl > hControl; P<0.01). For the Mattis Dementia Rating Scale, the three groups showed a significant change over time (F(1,89)=5.070; P<0.05). For the Rey-Osterrieth complex figure for visual memory, the pControl group performed worse than the hControl group in both immediate recall (18.7±7.1 vs 24.2±6.2; post-hoc analysis: pControl < hControl, F=6.135; P<0.01) and delay recall tasks (19.4±6.1 vs 23.5±5.5; post-hoc analysis: pControl < hControl, F=4.943; P<0.01). In the Hong Kong List Learning Test for verbal memory, the pRBD and pControl groups had a poorer performance than the hControl group, but there was no significant difference between the pRBD and pControl group.

Neuroimaging findings

Thirty participants completed the triple neuroimaging study (14 pRBD patients, 6 pControls, and 10 hControls). The three groups were comparable in terms of age and sex. There was no significant difference in dopamine transmission (F-DOPA or Raclopide scan) among the three groups.

Discussion

The incidence of PD among pRBD patients was 1.1%. We could not conduct further analysis to explore the predictors for the conversion as the case number was small. Nonetheless, the two patients who developed PD were male and had onset of RBD symptoms after the age of 50 years. A longer term follow-up of this clinical cohort may help to identify potential predictors. The incidence of developing RBD in psychiatric patients was 1.9%.

RBD symptoms were persistent in about 80% of pRBD patients, even though almost 60% of them were prescribed symptomatic drug treatment. They had persistently higher REM-related EMG muscle activities at follow-up, compared with their control counterparts. Compared with pControls, pRBD patients had persistently more depressive and anxiety symptoms. Moreover, they persistently scored higher on the Unified Parkinson's Disease Rating Scale at baseline and follow-up, and the magnitude of increase with time was higher in pRBD patients than their control counterparts. The findings suggest that pRBD patients might harbour more subtle motor symptoms of Parkinsonism, although the severity of these symptoms did not reach the threshold for a clinical diagnosis of PD.

Various neurocognitive abnormalities have been identified as early markers for neurodegeneration in patients with idiopathic RBD. Nonetheless, the validity of these markers has not been examined in patients with co-morbid RBD and MDD. As patients with MDD are known to have neurocognitive deficits, there is a need to establish the neurocognitive profile of co-morbid RBD and MDD. We found no significant difference between pRBD patients and pControls in most neurocognitive tests, including those for memory, attention, and executive functions. Those with RBD symptoms were more likely to show persistent olfactory dysfunction. Olfactory function might be a more reliable marker in patients with co-morbid RBD and MDD. For dopamine transmission, we found no significant differences among the groups.

The study was limited by a small sample size to determine predictors for the conversion of neurodegeneration.

Conclusion

RBD in psychiatric patients may run a persistent course with a high prevalence of sleep-related injury despite symptomatic treatment. Similar to typical RBD in elderly patients, pRBD patients also had a higher chance of developing neurodegeneration. Compared with pControls, pRBD patients had more prominent symptoms of depression and anxiety and increased motor symptoms of Parkinsonism, and were more likely to have olfactory dysfunction and demonstrate a persistent increase in REM-related EMG activities.

Symptoms of RBD are prevalent and persistent in psychiatric patients. Patients with pRBD are associated with a higher prevalence of sleep-related injuries to themselves and bed-partners despite symptomatic drug treatment. In addition, longitudinal follow-up revealed a higher prevalence of persistent olfactory dysfunction and increased motor symptoms of Parkinsonism in pRBD patients.

Acknowledgements

This study was supported by the Health and Medical

Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120326). We dedicate this report to all the participants. The project could not have succeeded without their cooperation and participation.

Ethical Approval

The study was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee (Ref No: CRE-2012.577).

Declaration

The authors have no conflicts of interest to disclose.

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Association of serum folate level with toxicity of capecitabine in patients with colorectal cancers: a prospective cohort study

SL Chan*, BBY Ma, AKC Chan

KEY MESSAGES

1. A higher serum folate level is associated with a higher rate of moderate-to-severe toxicity of capecitabine in patients with colorectal cancer.
2. The safety profile of capecitabine is similar between Chinese and western populations, except for a lower rate of diarrhoea and hand-foot skin reactions in the Chinese population.
3. Future studies are needed to determine whether higher folate intake is associated with a worse toxicity profile during capecitabine treatment in cancer patients.

Hong Kong Med J 2018;24(Suppl 3):S29-31
HMRF project number: 01120226

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Introduction

Colorectal cancer is the most common cancer in Hong Kong; over 4000 new cases were diagnosed in 2013.[1] Surgery is the only curative treatment. Adjuvant chemotherapy is indicated in stage III and high-risk stage II disease to reduce the risk of recurrence and distant metastasis after surgery. For stage IV disease, chemotherapy is the mainstay of treatment for palliation. There is a trend of replacing the chemotherapy drug 5-fluorouracil (5-FU) with an oral agent, capecitabine, which is a prodrug of 5-FU that undergoes enzymatic activation in the liver to become the active metabolite.[2] Capecitabine has been recommended in various international guidelines as a single agent or in combination with other chemotherapy agents for colorectal cancer. During capecitabine treatment, the rate of toxicity differs between western and Asian patients. A higher serum folate level has been associated with a higher rate of toxicity in western populations. We conducted a prospective study to examine the association of serum folate level with toxicity during capecitabine treatment in local Chinese patients.

Methods

A prospective cohort study design was used. We hypothesised that a higher serum folate level was associated with an increased rate of moderate-to-severe toxicity in patients who underwent capecitabine treatment for colon cancer. A total of 140 patients with a diagnosis of colorectal cancer who were scheduled to undergo capecitabine or capecitabine-oxaliplatin treatment at the Department of Clinical Oncology of the Prince of Wales Hospital were invited to participate. Informed consent was obtained from each participant before commencement of capecitabine treatment. At baseline, serum and red blood cell folate levels were assessed before commencement of chemotherapy. Patients underwent routine clinical follow-up every 3 weeks. Additional follow-up was provided to those who developed toxicity and required a dose delay. In accordance with the Common Terminology Criteria for Adverse Events 4.0,[3] the type and grade of toxicity was documented on the clinical record form at every visit. Patients were given capecitabine 1250 mg/m^2 twice daily (or 1000 mg/m^2 twice daily when combined with oxaliplatin 100 mg/m^2) for 14 days every 3 weeks. For patients with mild-to-moderate renal impairment (creatinine clearance, 30-50 mL/min), capecitabine was administered at a 25% dose reduction. Patients who developed toxicity secondary to capecitabine treatment were managed in accordance with standard departmental procedures.

Results

A total of 193 patients were recruited from October 2013 to September 2015, of whom 144 were eligible. The median age was 60 (range, 55-68) years. More than 95% of patients had an Eastern Cooperative Oncology Group performance status of 0 (asymptomatic) or 1 (symptomatic but completely ambulatory). About 70% of patients received capecitabine-based treatment. Most (57.3%) had stage III colorectal cancer, and most (74.3%) underwent capecitabine treatment in combination with other agents.

In terms of toxicity of grade 2 or higher, nausea was the most common (47.9%), followed by palmar-plantar erythrodysesthesia (25.0%) and diarrhoea (23.7%) [Table 1]. The rate of grade 3 or higher toxicities was lower than 5%, except for the rate of grade 3 diarrhoea (5.6%). There was no treatment-related death. A total of 32 (23.5%) patients required dose reduction of capecitabine.

TABLE 1. Grade and rate of capecitabine-related toxicity

Grade of toxicity	No. (%) of patients (n=144)	No. (%) patients with ≥ grade 2 toxicity
Nausea		69 (47.9)
0	35 (24.3)	
1	40 (27.8)	
2	66 (45.8)	
3	3 (2.1)	
Vomiting		18 (12.5)
0	82 (56.9)	
1	44 (30.6)	
2	13 (9.0)	
3	5 (3.5)	
Diarrhoea		35 (23.7)
0	65 (45.0)	
1	45 (31.3)	
2	26 (18.1)	
3	9 (5.6)	
Stomatitis		19 (13.2)
0	61 (42.4)	
1	64 (44.4)	
2	15 (10.4)	
3	4 (2.8)	
Skin hyperpigmentation		8 (5.5)
0	27 (18.8)	
1	109 (75.7)	
2	8 (5.5)	
Palmar-plantar erythrodysesthesia		36 (25.0)
0	49 (34.0)	
1	59 (41.0)	
2	35 (24.3)	
3	1 (0.7)	
Skin ulceration		2 (1.4)
0	129 (89.6)	
1	13 (9.0)	
2	2 (1.4)	
Neutropaenia		29 (20.1)
0	100 (69.4)	
1	14 (9.7)	
2	24 (16.7)	
3	5 (4.2)	

The mean serum folate level was 27.7 nmol/L (range, 12.8-45.4 nmol/L). When the cut-off value at the 75th percentile (33.9 nmol/L) was used, 36 (26.4%) patients belonged to the high serum folate group. The mean red blood cell folate level was 1958.4 nmol/L (range, 1183-3716 nmol/L). At the cut-off value of the 75th percentile at 2212.5 nmol/L, 36 (25.0%) patients belonged to the high red blood cell folate group (Table 2).

Univariable analysis showed that serum folate level (not red blood cell folate level) was predictive of toxicity of grade 2 or higher (odds ratio [OR]=1.069, 95% confidence interval [CI]=1.015-1.126; P=0.011; Table 3). Multivariable analysis by logistic regression showed that both alkaline phosphatase level (OR=0.992, 95% CI=0.983-1.000; P=0.062) and serum folate level (OR=1.061, 95% CI=1.007-1.117; P=0.027) were independent predictors of grade 2 or higher toxicity (Table 3).

Discussion

We have demonstrated that serum folate level is a modest predictor of moderate-to-severe toxicity related to capecitabine-based chemotherapy. Our results are consistent with previous findings that only serum folate level (not red blood cell folate level) is associated with moderate-to-severe toxicity during a capecitabine-based regimen.[4,5] The mechanism by which serum folate levels affect 5-FU-related toxicity remains unclear. One postulation is that 5-FU relies on the presence of reduced folate to bind to one of the target enzymes, thymidine synthase, for action. High serum folate level is reflective of the recent intracellular reduced folate level in normal cells around the time of chemotherapy, which may lead to more toxicity.[4] In contrast, red blood cell folate level is more closely related to the average long-term level of folate in the cells. Hence, its level is not directly related to chemotherapy-related toxicity. This finding suggests that serum folate level, instead of red blood cell folate level, should be used in future research of 5-FU related toxicity.

This study represents the largest prospective series so far on the safety profile of capecitabine-based chemotherapy in a Chinese population. It was reassuring that capecitabine-based chemotherapy was generally tolerable in our Chinese cohort: no treatment-related deaths and <7% of toxicities of grade 3 or above were recorded.[6,7] Compared with clinical trials of capecitabine in the western population, our Chinese cohort had less severe diarrhoea (6.6% vs 10%) and palmar-planter erythrodysesthesia (<1% vs >15%). The serum folate

TABLE 2. Serum and red blood cell folate concentrations

Blood fraction	Folate concentration, nmol/L			No. (%) of patients	
	Mean ± standard deviation	Median (range)	75th percentile cut-off value	Low level	High level
Serum	27.7±9.0	26.5 (12.8-45.4)	33.9	108 (73.6)	36 (26.4)
Red blood cell	1958.4±492.0	1882.0 (1183-3716)	2212.5	108 (75.0)	36 (25.0)

TABLE 3. Univariable and multivariable analyses for predictors of toxicity of grade 2 or higher

Variable	Univariable analysis		Multivariable analysis	
	Odds ratio (95% confidence interval)	P value	Odds ratio (95% confidence interval)	P value
Age	0.956 (0.917-0.997)	0.0359	-	-
Sex (male)	0.638 (0.286-1.425)	0.2729	-	-
Eastern Cooperative Oncology Group status	0.742 (0.342-1.608)	0.4493	-	-
Body weight	0.977 (0.944-1.011)	0.1759	-	-
Tumour-node-metastasis classification	0.639 (0.134-3.050)	0.5748	-	-
Treatment: palliative	0.687 (0.296-1.598)	0.3838	-	-
Creatinine clearance	1.006 (0.988-1.024)	0.5346	-	-
Bilirubin	0.974 (0.885-1.065)	0.5288	-	-
Albumin	1.049 (0.934-1.177)	0.4211	-	-
Alanine aminotransferase	1.006 (0.980-1.032)	0.6473	-	-
Alkaline phosphatase	0.991 (0.982-0.999)	0.0298	0.992 (0.983-1.000)	0.0616
Serum folate	1.069 (1.015-1.126)	0.0109	1.061 (1.007-1.117)	0.0268
Red blood cell folate	1.001 (1.000-1.002)	0.1387	-	-

level may be a contributing factor to the geographical difference in toxicity of 5-FU.

Conclusion

There is a potentially negative effect of serum folate on capecitabine-related toxicity. Patients should be advised against taking extra vitamin supplements or making dramatic changes to their consumption of vegetables during chemotherapy.

Acknowledgement

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120226).

Ethical Approval

The study was approved by the Joint Chinese University of Hong Kong - New Territories East Cluster Clinical Research Ethics Committee (Ref. No: CRE-2013.019). Patient consent for participation was obtained.

Declaration

The authors have no conflicts of interest to disclose.

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Vitamin D deficiency among healthy infants in Hong Kong: a pilot study

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KEY MESSAGES

1. This pilot study suggests that vitamin D deficiency is prevalent among Chinese infants in Hong Kong.
2. Exclusive breastfeeding is an important factor associated with vitamin D deficiency.
3. More studies are needed to evaluate the health implications of vitamin D deficiency during early infancy.

Hong Kong Med J 2018;24(Suppl 3):S32-5
HMRF project number: 01120026

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Introduction

A suboptimal vitamin D level has been associated with various disease conditions, although whether a causal relationship exists remains uncertain.[1] Vitamin D insufficiency among children has become a worldwide issue.[2] In infants, it could be attributed to exclusive breastfeeding and limited sunlight exposure.[3] 25-Hydroxyvitamin D, also known as 25(OH)D, is the main circulating form of vitamin D and is used to define vitamin D status. A consensus on the serum 25(OH)D concentration that should define vitamin D deficiency for infants and children has not yet been reached. If bone health is used as the clinical outcome, vitamin D deficiency is generally defined as a serum 25(OH)D concentration of <50 nmol/L, at which bone adverse outcomes are observed. In Hong Kong, efforts to promote breastfeeding have successfully boosted the rate at 3 months from 10% in 1997-2000 to 37% in 2010.[4] Although more children benefit from the advantages of breast milk, there may be a growing risk of vitamin D deficiency among exclusively breastfed children. There is no local recommendation about postnatal vitamin D supplementation, and updated local data on this issue are needed. This pilot study aimed to explore the prevalence of vitamin D deficiency among healthy infants in Hong Kong.

Methods

Healthy Chinese newborns were recruited in the postnatal wards of the Prince of Wales Hospital with convenience sampling. Mothers of eligible infants were asked to participate and written informed consent was obtained. When the infants reached 3 months of age, they and their parents were invited to return for the measurement of infants' serum vitamin D level and completion of questionnaires. Infants were excluded from analysis if they had congenital anomalies, renal or hepatic diseases, feeding problems and/or malabsorption, metabolic bone disease or calcium disorder, rickets, or hypocalcaemia; if they were taking medications known to affect plasma 25(OH)D concentration; or if the mother had been treated with vitamin D within 6 months of the current pregnancy.

Anthropometric measurements of infants' recumbent length and weight were made. For serum 25(OH)D measurement, 1 mL of blood was collected, centrifuged, aliquoted, and stored at -70°C until analysis. The sum of $25(OH)D_3$ and $25(OH)D_2$, hence the total serum 25(OH)D, was measured by tandem mass spectrometry after ultra-performance liquid chromatography separation using a pentafluorophenyl column (UPLC-Xevo TQ System; Waters Corporation, Milford [MA], USA). This method separated out the biologically inactive 3-epi-$25OHD_3$, which has been reported to be present in infants in a significant amount.[5]

Parametric, non-parametric, and categorical data were presented as mean with standard deviation (SD), median with interquartile range (IQR), and percentage, respectively. The prevalence of vitamin D deficiency was calculated. The vitamin D sufficient and deficient groups were compared using the chi-square test for categorical variables and Mann-Whitney *U* test and *t* test for non-parametric and parametric variables, respectively. Spearman's rho was used to test the correlation of serum 25(OH)D level with breastfeeding duration, growth variables at 3 months, and plasma alkaline phosphatase, calcium, and phosphorous concentrations. A P value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS (Windows version 20.0; IBM Corp, Hong Kong).

Results

Of 269 infants enrolled, 155 (of whom 75 were male) completed the study, giving a response rate of 57.6%. Baseline characteristics between those who completed the study and those who dropped out did not differ significantly in terms of sex distribution, gestational age, birthweight, season at birth, maternal age, maternal smoking status, or feeding practice at recruitment (Table 1).

Serum 25(OH)D concentration was determined at a mean age of 98.3 (SD, 9.7) days. No infant received vitamin D supplementation. The median serum 25(OH)D concentration at 3 months was 58 (IQR, 32-75) nmol/L. Of 155 infants, 52 (33.5%) had vitamin D deficiency defined as a serum 25(OH)D concentration of <50 nmol/L; of these, 34 (21.9%) had a concentration of <25 nmol/L, indicating severe deficiency. The proportion of infants with a history of exclusive breastfeeding was higher in those with vitamin D deficiency than in those with vitamin D sufficiency (P<0.001, Table 2). The duration of exclusive breastfeeding was inversely correlated with the serum 25(OH)D concentration at 3 months of age (r= −0.605, P<0.001). Thirty eight (24.5%) of the infants were exclusively breastfed at 3 months, 37 (97.4%) had vitamin D deficiency, with a mean concentration of 19.7 (SD, 14.3) nmol/L. The serum 25(OH)D concentration was positively correlated with plasma phosphorous concentration (r=0.556, P<0.001). The serum 25(OH)D concentration was not correlated with infant exposure to sunlight, plasma calcium or alkaline phosphatase concentration, or growth at 3 months of age.

Discussion

Our pilot study suggests that vitamin D deficiency may be prevalent in local Chinese infants at 3 months of age. Exclusive breastfeeding is associated with vitamin D deficiency; the duration of exclusive breastfeeding is inversely correlated with the serum 25(OH)D concentration at 3 months of age. Among exclusively breastfed infants, 97.4% had vitamin D deficiency; the rate is higher than that reported in other studies (6% to 81%).[6-8] The difference may be due to variation among different localities and ethnicities; endogenous production of vitamin D may be influenced by ethnic, cultural, and environmental factors such as skin pigmentation, extent of sun exposure, and geographic latitude. The prevalence of vitamin D deficiency in our cohort at 3 months was 33.5%, which is higher than the 29.8% and 28.3% reported in studies that included older infants and defined vitamin D insufficiency as a serum 25(OH) D concentration of <75 nmol/L.[9,10] The differences indicate that serum 25(OH)D concentrations may vary during infancy, and infants at 3 months may be at a higher risk of vitamin D deficiency than older infants. Vitamin D obtained through transplacental passage from the mother stores in the newborn and lasts until around 3 months.[11] At 3 months, infants rely on breast milk and/or formula milk as their sole source of nutrients. It is also the age at which there is limited sunlight exposure. Exhaustion of the body's reserve of vitamin D and lack of solid food and sun exposure may explain why exclusively breastfed infants have a high prevalence of vitamin D deficiency. When children get older and have a

TABLE 1. Baseline characteristics of participants and dropouts

Characteristic	Participants (n=155)*	Dropouts (n=114)*	P value
Males:females, No.	75:80	50:64	0.54
Gestational age at birth, w	38.9±1.1	39.4±5.8	0.25
Birth weight, kg	3.1±0.33	3.1±0.34	0.61
Season at birth, No.			0.43
Winter	31	21	
Spring	57	40	
Summer	46	42	
Autumn	21	11	
Maternal age, y	30.6±4.4	30.2±3.9	0.27
Maternal smoking during pregnancy, No. (%)	7 (4.5)	3 (2.6)	0.35
Feeding practice at recruitment, No.			0.49
Breastfeeding	68	56	
Formula milk	20	15	
Mixed	67	43	

* Data are presented as mean ± standard deviation, unless otherwise indicated

TABLE 2. Comparison of participants with vitamin D sufficiency or deficiency

Characteristic	Participants (n=155)*	Vitamin D sufficiency (n=103)*	Vitamin D deficiency (n=52)*	P value
Serum 25-hydroxyvitamin D, nmol/L	58 (32-75)	70 (58-80)	18 (12-32)	<0.001
Males:females, No.	75:80	49:54	26:26	0.78
Age, d	98.3±9.7	97.4±8.8	100.1±11.2	0.13
Gestation at birth, w	38.9±1.1	38.8±1.1	39.0±1.2	0.35
Birth weight, kg	3.1±0.3	3.2±0.3	3.1±0.3	0.47
Season at birth, No.				0.57
Winter	31	18	13	
Spring	57	39	18	
Summer	46	30	16	
Autumn	21	16	5	
Season at sampling, No.				0.74
Winter	21	15	6	
Spring	32	19	13	
Summer	53	37	16	
Autumn	49	32	17	
History of exclusive breastfeeding, No. (%)	73 (47.1)	29 (28.2)	44 (84.6)	<0.001
Breastfeeding ever, No. (%)	108 (69.7)	-	-	-
Maternal age, y	30.5±4.2	30.6±4.4	30.2±3.9	0.27
Nutritional supplement during pregnancy, No. (%)	132 (85.2)	86 (83.5)	45 (86.5)	0.63
Nutritional supplement during lactation among mothers who ever breastfed, No./total (%)	82/108 (75.9)	25/64 (39.1)	22/44 (50.0)	0.17
Maternal smoking during pregnancy, No. (%)	7 (4.5)	6 (5.8)	1 (1.9)	0.48
Maternal sunlight exposure during pregnancy, h/w	1.5 (0.5-5.44)	1.75 (0.5-5.9)	1.3 (0.5-3.5)	0.36
Infant sunlight exposure, h/w	1 (0-3)	1 (0-2.8)	1.25 (0-4)	0.12
Sunblock use during pregnancy, No. (%)	68 (43.9)	42 (40.8)	25 (48)	0.49
Sunblock use for infants, No. (%)	6 (3.9)	3 (2.9)	3 (5.8)	0.41
Household income <HK$20 000, No. (%)	31 (20)	16 (15.5)	15 (28.8)	0.09
Body weight at 3 months, kg	6.1±0.7	6.1±0.7	6.1±0.7	0.70
Body height at 3 months, cm	61.5±2.3	61.4±2.4	61.7±2.0	0.50
Plasma calcium, mmol/L	2.6±0.1	2.6±0.1	2.6±0.1	0.12
Plasma phosphorous, mmol/L	1.8±0.2	1.9±0.1	1.7±0.2	<0.001
Plasma alkaline phosphatase, IU/L	283.8±75.5	283.6±71.0	284.2±84.6	0.96

* Data are presented as mean ± standard deviation or median (interquartile range), unless otherwise indicated

more diverse dietary intake and sunlight exposure, vitamin D status may improve. Longitudinal studies are required to evaluate the natural change in vitamin D status during infancy.

Exclusive breastfeeding is consistently associated with vitamin D deficiency.[12,13] The vitamin D content of breast milk is low, even in vitamin D–adequate lactating mothers.[12,13] The major source of vitamin D is from sun exposure and diet, so exclusively breastfed infants with limited sun exposure are at higher risk of vitamin D deficiency. In our study, there were no significant differences in growth or calcium and alkaline phosphatase levels in those who were vitamin D sufficient or deficient. Although plasma phosphorous level is associated with serum 25(OH)D concentration, its clinical significance remains uncertain. Studies to investigate the effect of vitamin D supplementation on bone health have not demonstrated any dose-response relationship between vitamin D concentration and bone mineral content or accretion.[14,15] Further Mendelian randomisation studies are needed to

investigate various clinical outcomes of vitamin D deficiency during pregnancy and early childhood and to define the optimal serum 25(OH)D concentration in infancy.

There are several limitations in our study. It was a single-centre pilot study with limited sample size and uncertain generalisability to the entire local population. The dropout rate was high. Vitamin D status was determined only at the age of 3 months. Maternal vitamin D status during pregnancy was not assessed and the clinical outcomes related to vitamin D deficiency were not studied. Nonetheless, this pilot study revealed a previously unrecognised proportion of infants with vitamin D deficiency. Interventional studies in infants to evaluate the clinical implications of vitamin D deficiency are needed to provide guidance for future studies and recommendations about vitamin D supplementation during pregnancy and infancy.

Conclusion

Vitamin D deficiency is prevalent in Hong Kong infants and is associated with exclusive breastfeeding. More studies are needed to evaluate the health implications of vitamin D deficiency during early infancy.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120026). Dr Chung-shun Ho from the Department of Chemical Pathology performed the measurement of serum 25(OH)D concentration. The authors thank all parents and children who participated in this study.

Ethical Approval

This pilot cohort study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CRE-2012.436). Parental consent for participation was obtained.

Declaration

The authors have no conflicts of interest to disclose.

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Reference range for brachial artery flow-mediated dilation in healthy Chinese children and adolescents

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KEY MESSAGES

1. This study establishes the local reference range for flow-mediated dilation in healthy Chinese children and adolescents (aged 8 to 18 years) in Hong Kong.
2. In females, flow-mediated dilation increased rapidly from 8.29% at age 8 years to reach a peak (8.80%) at age 13 years, then decreased to 8.38% at age 18 years.
3. In males, flow-mediated dilation increased gently from 8.34% at age 8 years to a peak (8.77%) at age 14 years, then decreased to 8.54% at age 18 years.

Hong Kong Med J 2018;24(Suppl 3):S36-8
HMRF project number: 01120406

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Introduction

The vascular endothelium was once considered an inert barrier that separates the vascular wall from the circulating blood. There is now evidence to suggest that this layer is active and functionally important in the maintenance of vascular homeostasis. Endothelial function can be assessed by measuring changes in the diameter of conduit arteries, commonly the brachial artery. Ultrasonography of flow-mediated dilation (FMD), also known as endothelium-dependent dilation, is the standard means to non-invasively evaluate vascular response. In adults, abnormal FMD of the brachial artery is an independent predictor of the development of cardiovascular adverse events.[1] In children, impaired FMD is associated with diabetes, low birthweight, dyslipidaemia, Henoch-Schönlein purpura, and sleep-disordered breathing.[2]

Few large-scale studies have been published on the reference range of FMD in children. The range of FMD differs between different ethnic and geographical populations. It is unknown if puberty has any effects on endothelial function. No such studies have been carried out in ethnic Chinese populations, and the use of a reference range for other ethnic groups may under- or over-diagnose endothelial dysfunction. We aimed to establish a reference range for FMD in Chinese children and adolescents in Hong Kong.

Methods

Ethnic Chinese children and adolescents (age, 8 to 18 years) were recruited from the community by the random selection of primary and secondary schools from four regions of Hong Kong (Hong Kong Island, Kowloon, New Territories East, and New Territories West) from May 2014 to July 2015. Anthropometric measurements included weight, height, body mass index, and waist circumference. After the participant had been laying in a recumbent position for 10 to 15 minutes, resting blood pressure was measured by oscillometry with the Datascope Accutorr Plus (Soma Technology, Bloomfield [CT], United States), which has been validated in local Chinese school children. For brachial artery FMD, a linear array transducer (L10-5 median frequency, 7.5 MHz) and MicroMaxx Ultrasound System (FUJIFILM SonoSite, Bothell [WA], United States) was used. B-mode ultrasonography was performed. Reactive hyperaemia was induced by inflation of a pneumatic tourniquet placed around the forearm (distal to the segment of the artery being scanned) to a pressure of 180 mm Hg for 4 to 5 minutes, followed by release to cause endothelium-dependent FMD. The artery was scanned for 30 seconds before and 90 seconds after deflation/release. FMD was defined as the percentage increase in baseline artery diameter after the corresponding stimulus, with reference to baseline. The absolute difference in artery diameter was recorded before and after stimulus. This is the most accepted and established method used in our unit, and the whole procedure results in minimal discomfort and is well tolerated.[3]

Results

Six primary schools and 14 secondary schools were recruited; two to three classes from each school were randomly assigned. Six participants were excluded

because of self-reported hypertension; none had any chronic cardiovascular diseases. Of the students, 36 (7%) of the primary and 158 (12%) of the secondary school students declined to participate but reasons were not recorded. A total of 480 primary school students and 1157 secondary school students were recruited. Their anthropometric measurements, baseline artery diameter, blood pressure, and FMD by age and sex are presented in Tables 1 and 2.

Analysis of correlation coefficient confirmed significant correlations of FMD with age (r=0.060) and height (r=0.066) but not with weight, waist circumference, body mass index, or baseline artery diameter. The extent of FMD increased with age in both sexes, and the increase plateaued after about age 14 years; this trend was more obvious in females than in males. In females, FMD increased rapidly from 8.29% to reach the peak (8.80%) at age 13 years, then decreased to 8.38% at age 18 years. In males, FMD increased gently from 8.34% to the peak (8.77%) at age 14 years, then decreased to 8.54% at age 18 years. The mean FMD in Hong Kong children aged 14 years (males, 8.77%; females, 8.70%) was higher than that reported in the United Kingdom (males, 7.46%; females, 7.27%) [all P<0.01, Table 3], although the mean FMD of children aged 9 to 11 years in both studies was almost identical.[4]

Discussion

This study is the largest cross-sectional study of FMD in children and adolescents. It establishes the reference range for FMD in Chinese children and adolescents in Hong Kong. In our study, the FMD of participants was not related to baseline vessel

TABLE 1. Anthropometric characteristics by age and sex

Age, y	No. of males	No. of females	Height, cm*		Weight, kg*		Waist circumference, cm*		Body mass index, kg/m²*	
			Males	Females	Males	Females	Males	Females	Males	Females
8	58	58	129.50±6.00	128.90±5.30	27.30±4.87	27.27±4.68	73.93±124.01	58.48±7.46	16.17±1.96	16.35±2.14
9	58	57	134.10±5.80	132.90±5.30	28.05±3.47	26.47±3.24	56.93±4.27	55.76±8.72	15.55±1.26	14.93±1.18
10	58	58	138.60±5.30	139.80±6.30	31.64±4.82	31.76±4.36	60.30±5.72	59.55±5.06	16.39±1.69	16.22±1.51
11	58	58	145.80±6.20	148.80±6.80	35.71±5.51	37.05±5.33	61.40±5.66	61.59±6.08	16.71±1.70	16.68±1.71
12	99	99	154.20±7.30	154.20±5.40	47.79±11.71	46.83±9.05	69.22±10.55	67.70±7.19	19.91±3.79	19.64±3.33
13	101	99	160.50±8.30	157.00±4.90	48.65±8.26	47.00±6.10	66.97±6.57	68.49±6.52	18.79±2.23	19.02±2.02
14	100	96	166.40±6.40	158.90±5.80	54.94±7.45	51.42±8.85	79.07±93.17	68.91±8.17	19.83±2.43	20.33±3.06
15	88	80	168.90±7.70	159.10±6.20	56.32±8.58	55.36±11.70	80.29±99.33	70.87±10.30	19.68±2.38	21.91±4.78
16	88	89	170.30±6.50	159.60±5.50	61.43±11.84	49.17±5.60	72.48±12.79	68.61±6.68	21.16±3.69	19.29±1.93
17	74	81	170.70±5.80	160.00±5.90	63.42±13.56	52.92±8.70	75.63±11.99	70.99±7.99	21.74±4.39	20.65±2.88
18	47	33	170.60±6.20	159.20±5.50	64.81±15.17	51.98±7.07	75.91±14.34	68.07±7.56	22.21±4.71	20.49±2.32

* Data are presented as mean ± standard deviation

TABLE 2. Flow-mediated dilation, baseline artery diameter, and blood pressure by age and sex

Age, y	No. of males	No. of females	Flow-mediated dilation, %*		Baseline artery diameter, mm*		Systolic blood pressure, mm Hg*		Diastolic blood pressure, mm Hg*	
			Males	Females	Males	Females	Males	Females	Males	Females
8	58	58	8.34±0.87	8.29±0.82	2.25±0.23	2.14±0.2	103.11±9.58	103.05±9.8	60.95±8.4	60.64±7.59
9	58	57	8.65±0.67	8.64±0.79	2.30±0.25	2.11±0.25	104.40±9.3	104.37±9.72	62.67±7.3	62.40±6.93
10	58	58	8.44±0.96	8.37±0.7	2.42±0.25	2.26±0.21	103.94±9.48	104.99±10.28	61.46±7.38	62.90±8.62
11	58	58	8.50±0.74	8.33±0.99	2.48±0.3	2.30±0.27	103.64±9.26	107.25±9.75	62.30±7.86	61.17±7.3
12	99	99	8.32±1.07	8.64±0.76	2.69±0.28	2.46±0.24	110.72±9.23	107.54±9.87	63.97±7.07	61.82±6.83
13	101	99	8.53±1.11	8.80±0.81	2.83±0.41	2.46±0.26	108.73±10.15	105.65±8.25	62.33±8.32	62.06±5.98
14	100	96	8.77±0.89	8.70±0.91	2.93±0.32	2.52±0.24	115.27±9.26	108.30±8.21	65.29±6.99	62.34±7.18
15	88	80	8.65±0.98	8.64±0.68	2.97±0.29	2.56±0.29	114.95±10.63	107.95±8.77	65.41±7.83	62.85±8.16
16	88	89	8.63±0.99	8.63±0.7	2.95±0.31	2.47±0.23	118.32±11.59	106.63±10.58	68.55±9.05	63.38±7.49
17	74	81	8.37±1.22	8.64±0.72	3.03±0.52	2.49±0.3	118.23±10.51	109.65±9.55	69.69±6.68	65.15±7.66
18	47	33	8.54±1.09	8.38±0.97	3.08±0.37	2.48±0.23	119.99±8.57	110.13±9.19	70.23±8.22	67.77±6.83

* Data are presented as mean ± standard deviation

TABLE 3. Flow-mediated dilation by age in Hong Kong children versus United Kingdom children[4] (all P<0.01)

Age, y	Hong Kong children						United Kingdom children[4]					
	No. of males	No. of females	Flow-mediated dilation, %*		Adjusted flow-mediated dilation, %*		No. of males	No. of females	Flow-mediated dilation, %*		Adjusted flow-mediated dilation, %*	
			Males	Females	Males	Females			Males	Females	Males	Females
8	58	58	8.34±0.87	8.29±0.82	8.34±0.05	8.29±0.04	29	11	7.90±3.45	6.29±3.20	10.23±7.13	13.89±7.23
9	58	57	8.65±0.67	8.64±0.79	8.65±0.05	8.64±0.05	43	23	7.90±3.30	8.15±3.38	8.03±6.88	9.30±7.11
10	58	58	8.44±0.96	8.37±0.7	8.44±0.05	8.37±0.04	65	68	8.33±4.01	9.15±4.54	9.56±6.97	9.57±7.13
11	58	58	8.50±0.74	8.33±0.99	8.50±0.06	8.32±0.06	78	72	8.08±3.49	8.49±4.29	8.31±6.98	9.25±6.95
12	99	99	8.32±1.07	8.64±0.76	8.32±0.06	8.64±0.05	53	53	7.42±3.58	7.95±3.75	8.13±7.09	7.79±6.85
13	101	99	8.53±1.11	8.80±0.81	8.53±0.08	8.80±0.05	40	51	7.86±2.76	8.02±3.75	8.10±7.05	8.52±7.03
14	100	96	8.77±0.89	8.70±0.91	8.77±0.07	8.70±0.05	46	33	7.46±3.20	7.27±3.55	7.98±7.37	6.25±6.96
15	88	80	8.65±0.98	8.64±0.68	8.65±0.06	8.64±0.06	42	35	6.89±2.94	7.45±3.18	6.78±23.36	7.84±7.17
16	88	89	8.63±0.99	8.63±0.7	8.64±0.06	8.63±0.05	34	36	7.47±4.15	8.71±4.22	7.05±6.90	9.43±7.16
17	74	81	8.37±1.22	8.64±0.72	8.38±0.11	8.69±0.06	30	21	6.71±3.33	9.82±4.45	5.98±6.85	8.55±7.46
18	47	33	8.54±1.09	8.38±0.97	8.54±0.08	8.38±0.05	32	22	6.06±2.84	8.64±2.80	6.61±7.11	9.94±7.16

* Data are presented as mean ± standard deviation

size. Although the mean FMD was approximately 8%, it increased with age and was distinct between males and females, indicating age- and sex-specific endothelial function. Other studies have reported significantly higher FMD values in females than in males.[4,5] Our results showed that the FMD value was higher in females at the ages of 12 and 13 years only. This finding may be related to the earlier onset of puberty in females than in males; most females were at a post-pubertal stage after 14 years of age. As a higher oestrogen level is associated with enhanced vascular function, oestrogen production may account for the sudden improved endothelial function in girls during puberty.[4]

Strengths of the study include a large sample size, balance between males and females in each age-group, and the uniform approach and analysis of FMD. Limitations of the study include a lower-than-expected sample size for the 17- to 18-year age-group. After the citywide university admission reform in 2012, most of this age-group would have left secondary schooling to enter tertiary education. We did not examine vasodilatation independent of the endothelium with sublingual nitroglycerine, as this would have prolonged the testing time and may have caused discomfort to our participants. Further longitudinal studies are needed, as there is no scientific evidence to suggest that improvement in endothelial function during childhood will translate into decreased risk of future cardiovascular diseases.

Conclusion

Our study established the reference range for FMD in healthy Chinese children and adolescents in Hong Kong.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120406). The authors thank all participants and their parents who participated in the study.

Ethical Approval

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC-2012.456). Written informed consent was obtained from the parents of each participant.

Declaration

The authors have no conflicts of interest to disclose.

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Association between childhood primary snoring and cardiovascular health

CT Au*, P Chook, AM Li, RYT Sung, MHM Chan, YK Wing

KEY MESSAGES

1. Although persistent primary snoring (PS) over an average of 5 years was associated with reduced endothelial function compared with controls, neither incidence nor resolution of PS was associated with significant changes in endothelial function.
2. These findings suggest no causal relationship between PS and increased cardiovascular risk in children.
3. Participants with incident obstructive sleep apnoea (OSA) had a higher ambulatory blood pressure than those without OSA. This suggests that OSA may increase the cardiovascular risk of children.

Hong Kong Med J 2018;24(Suppl 3):S39-40
HMRF project number: 01120736

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Introduction

Primary snoring (PS) is defined as habitual snoring but with no more than one respiratory event per hour during sleep, on the basis of overnight polysomnography. It is the mildest form of sleep-disordered breathing and is considered a benign condition that results in no complications and hence can be left untreated.[1] Nonetheless, studies have reported that PS in children is associated with adverse health outcomes including behavioural problems,[2] metabolic impairment,[3] and cardiovascular complications.[4] A previous study revealed that childhood PS is independently associated with poorer endothelial function.[4] Nonetheless, these cross-sectional studies could not provide any evidence of a causal relationship. Childhood PS may progress or resolve with growth.[5] Studies of a longitudinal association between PS and health outcomes are necessary to provide evidence of a causal relationship. If cardiovascular complications are found to worsen in children with persistent PS, a change in the current PS management paradigm may be needed.

Methods

We prospectively followed up two cohorts of children (one with PS and one without PS) to investigate the longitudinal association between PS and cardiovascular outcomes—namely, endothelial function and ambulatory blood pressure. We hypothesised that persistent PS in children was associated with reduced endothelial function and elevated ambulatory blood pressure, compared with non-snoring controls. All participants underwent overnight polysomnography and measurement of flow-mediated dilation (FMD) of the brachial artery (a measure of endothelial function) at baseline and at follow-up after a mean interval of 5 years. At the follow-up visit, 24-hour ambulatory blood pressure monitoring was also performed.

Results and Discussion

A total of 96 primary snorers and 111 non-snorers were analysed. The primary snorers had significantly lower FMD at both baseline (8.2%±1.3% vs 8.5%±1.0%; P=0.037) and follow-up (8.2%±0.9% vs 8.5%±0.8%; P=0.002). At follow-up, 76 of 96 snorers had persistent sleep-disordered breathing, whereas 73 of 111 non-snorers remained snoring-free.

The persistent sleep-disordered breathing group had non-significantly lower FMD at baseline (8.2%±1.2% vs 8.6%±0.9%; P=0.061) and significantly lower FMD at follow-up (8.3%±0.9% vs 8.6%±0.8%; P=0.026). Nonetheless, there was no significant difference in the changes in FMD between the two groups (0%±0.9% vs 0%±0.8%; P=0.9). Further analysis revealed that new-onset OSA at follow-up was associated with higher ambulatory blood pressure.

This study had several limitations. The diagnosis of PS was based on parental reports. The sample size was too small to have sufficient power in subgroup analyses to explore the predictors and outcomes of different courses of disease progression over time. The control group was not sex-matched with the PS group.

Conclusions

This study demonstrated that persistent PS in children was not associated with reduced endothelial function (increased cardiovascular risk) over an average of 5 years. These findings suggest no causal relationship between PS and increased cardiovascular risk in children. Nonetheless, a proportion of children were found to have incident OSA, which was associated with a higher ambulatory blood pressure. Children with PS should be followed up regularly to monitor its possible progression and to prevent any possible cardiovascular complications. Primary snoring can be a subtle but chronic problem; longitudinal studies with a longer follow-up period and regular assessments are needed to determine the association of childhood PS with any cardiovascular events in adulthood.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01120736). We thank the nurses and health care assistants in wards 11K and 11L of the Prince of Wales Hospital for their assistance during admission of participants. We also appreciate the active participation of all participants and their parents.

Declaration

The authors have no conflicts of interest to disclose.

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Evaluation of a novel clinicopathological marker *JK-1* for human oesophageal carcinoma

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KEY MESSAGE

The expression level of *JK-1* in oesophageal squamous cell carcinoma may serve as a clinicopathological marker for tumour differentiation, metastasis, and patient survival.

Hong Kong Med J 2018;24(Suppl 3):S41-4
HMRF project number: 01121886

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Introduction

Oesophageal cancer ranks the ninth most common malignancy and the sixth most frequent cause of cancer death in the world, with geographic variations in incidence and histological subtypes. In China, oesophageal squamous cell carcinoma (ESCC) accounts for >90% of the total incidence of oesophageal cancer; approximately 300 000 new cases of oesophageal cancer are diagnosed every year in the world of which almost half originate in the high-incidence regions of China.[1] According to the Hong Kong Cancer Registry in 2012, ESCC ranked the tenth most frequent cancer death among all other cancers for both sexes. The current treatment modalities for ESCC achieve relatively suboptimal survival and cure rates.[2] To further improve the management of this disease, novel prognostic markers for ESCC need to be identified.

Gene amplification and overexpression have been suggested to be the major genomic aberrations involved in the pathogenesis of ESCC. Previously, our group reported a novel oncogene *JK-1* in ESCC located in the chromosomal region 5p15.1-2; it frequently shows amplification in ESCC and other solid tumours.[3] Our collaborators also reported the overexpression of *JK-1* mRNA in colorectal tumours, providing the first evidence of the significance of *JK-1* mRNA overexpression in gastrointestinal cancer.[4] Nonetheless, there are no studies of *JK-1* protein expression in ESCC or its correlation with clinicopathological features.

Identifying novel histopathologial tumour markers for ESCC is important. Correlating the levels of these protein signals in tissues with the respective clinicopathological features enables better management of the disease. Detection of oncoprotein *JK-1* level in ESCC may improve the current protocols for cancer detection, prognosis, and treatment in the long run. This study may provide the ground work for future application of *JK-1* level detection in other human cancers.

Methods

A total of 303 paraffin-embedded archival paired samples of tumour and non-tumour tissues of ESCC with clinicopathological data collected after oesophagectomy since June 1996 at Queen Mary Hospital, Hong Kong were included for construction of the tissue microarray. The selection of tumour areas was assisted by an experienced histopathologist. Clinicopathological data were available for correlation with the detection of *JK-1* protein level in tissues. Moreover, 16 biopsy samples including one normal control of oesophageal epithelia were collected to assess the feasibiltiy of detecting *JK-1* protein level with dysplastic lesions or tumours.

Dewaxed paraffin sections (8 μm) of the tissue microarray from the ESCC archival cases comprising non-tumours and tumours or oesophageal biopsies were immunostained using the streptavidin-biotin-peroxidase complex method with the use of polyclonal antibody against the *JK-1* protein (Santa Cruz Biotechnology, USA). As pre-treatment, microwave-based antigen retrieval was performed in 10 mM citrate buffer (pH 6.0). Immunoreactions were visualised with diaminobenzidine, and the sections counterstained with 3% methylgreen. The NE1 cell-line blocks prepared from the *JK-1* transfected cells with overexpression of *JK-1* transcripts served as positive controls, and those without *JK-1* transfection as negative controls. In each section, five high-

TABLE 1. The clinicopathological features of the surgical specimens of oesophageal squamous cell carcinoma

Characteristics	All specimens (n=303)*	*JK-1* low expression (n=194)*	*JK-1* high expression (n=109)*	P value
Mean±SD patient age, y	64.19±8.71	63.81±6.19	65.67±7.44	0.476
Sex				0.409
Male	248 (81.8)	160	88	
Female	55 (18.2)	34	21	
Tumour depth				0.595
T1-3	238 (78.5)	157	81	
T4	65 (21.5)	37	28	
Lymph node metastasis				0.261
N0	122 (40.3)	75	47	
N1	181 (59.7)	119	62	
Distant metastasis				0.036
M0	253 (83.5)	156	97	
M1	50 (16.5)	38	12	
TNM stage				0.364
0/I/II	117 (38.6)	73	44	
III/IV	186 (61.4)	121	65	
Differentiation				0.005
Well & moderate	220 (72.6)	151	69	
Poor	83 (27.4)	43	40	

* Data are presented as No. (%) or No., unless otherwise stated

power fields were selected and a total of at least 700 cells were evaluated. The results were expressed as the percentage of cells with positive staining. The intensity of staining was estimated on a scale from 0 to 3 (negative, weak, moderate, and strong). The low expression group had scores of either 0 or 1, and the high expression group had scores of either 2 or 3.

The correlation between the expression level of *JK-1* and the clinicopathological features was analysed. The quantitative differences in protein expression in the different ESCC pathologic loci among *JK-1* positive cases was evaluated using the Chi-square test or Fisher's exact test. The association between *JK-1* protein expression and the patient's clinicopathological features was assessed using the Chi-square test. A P value of <0.05 was considered statistically significant.

Results

The expression level of *JK-1* was associated with tumour features in which the low-expression group was associated with less aggressive tumours with well and moderate differentiation (P=0.005) and absence of distant metastasis (P=0.036) [Table 1]. Survival was shorter in the high-expression than low-expression group (36.09 months vs 61.02 months, P=0.022, Fig]. Moreover, 14 (87.5%) of 16 cases with premalignant epithelia showed high expression of *JK-1*, suggesting the role of *JK-1* in early transformation of oesophageal epithelia (Table 2).

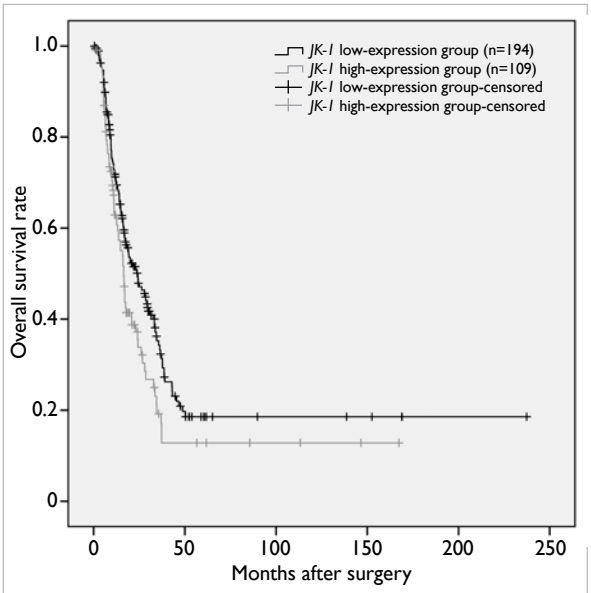

FIG. The overall 5-year survival of patients with oesophageal squamous cell carcinoma is higher in those with low expression than high expression of *JK-1* (P=0.022).

TABLE 2. Histopathological features of the 16 oesophageal biopsy samples

Patient sex/ age, y	Histological features	*JK-1* low expression	*JK-1* high expression
M/57	Squamous cell carcinoma		✓
M/75	Squamous cell carcinoma		✓
M/59	Squamous cell carcinoma		✓
M/59	Squamous cell carcinoma (in situ)		✓
M/72	Squamous cell carcinoma (in situ)		✓
M/76	Squamous cell carcinoma (poorly differentiated)		✓
F/62	Squamous cell carcinoma (moderately differentiated)		✓
M/74	Squamous cell carcinoma (moderately differentiated)		✓
M/57	Squamous cell carcinoma (moderately differentiated)	✓	
M/56	Squamous cell carcinoma (moderately differentiated)		✓
F/62	Minor dysplasia	✓	
M/57	Severe dysplasia		✓
M/76	Severe dysplasia		✓
M/57	Moderate dysplasia		✓
M/76	Moderate dysplasia	✓	
M/57	Normal epithelia	✓	

A similar pattern was observed in the oesophageal biopsy samples, although not statistically viable, in which a high expression of *JK-1* was detected in severe (2 out of 2) and moderate (1 out of 2) dysplastic lesions, whereas 9 out of 10 (90.0%) of the biopsy samples with tumours also showed high expression of *JK-1*.

Discussion

This is the first study to correlate the expression level of *JK-1* with the clinicopathological features of ESCC using the tissue microarray–immunohistochemistry method. Less-aggressive ESCC (well and moderately differentiated) and absence of distant metastasis were correlated with low-expression of *JK-1*, indicating the possibility of functional needs. This finding supports further investigation of the functional roles of *JK-1* in the process of molecular carcinogenesis in ESCC and other tumours, and development of treatments to target the *JK-1* protein using pharmacological or gene-therapy approaches. One example is the development of imatinib (Gilvec) that targets the tyrosine kinase functions of the *bcr-abl* fusion protein.[5] Moreover, the survival was shorter in samples with high-expression than low-expression of *JK-1* (36.09 months vs 61.02 months). High-expression of *JK-1* was also observed in 14 (87.5%) of 16 premalignant epithelia from surgical specimens, severe (2 out of 2) and moderate (1 out of 2) dysplastic lesions from biopsy samples, and 9 (90.0%) of 10 biopsy samples with early tumours. This suggests the possible involvement of *JK-1* in the early transformation of oesophageal epithelia. Similar observations were also reported for the role of DEC1 and connective tissue growth factors (CTGF and CCN2) in ESCC as prognostic markers for early detection.

Limitations of this study are the inaccessibility of a control population and lack of multivariate analysis that would involve observation and analysis of more than one variable at a time. Further studies should include analysis of other oncoproteins such as cyclooxygenase-2 that have been shown to have a close clinicopathological correlation with ESCC.

Conclusion

The findings of the present study provide the first evidence of the prognostic significance of *JK-1* expression in ESCC and may be beneficial to the management of ESCC. Extending the detection of *JK-1* expression in other cancers to study its prognostic significance is warranted.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#01121886). We thank Prof George SW Tsao, Department of Anatomy, The University of Hong Kong, for giving us the cell line NE1 as the control. We also thank Prof Alfred Lam, Griffith University, Australia, for providing expert advice for the present study.

Declaration

The authors have no conflicts of interest to disclose.

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Soluble fms-like tyrosine kinase-1 and placental growth factor in Chinese pregnant women during second and third trimesters

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KEY MESSAGE

The maternal soluble fms-like tyrosine kinase-1 to placental growth factor ratio in women with hypertensive disorder in pregnancy has prognostic value.

Hong Kong Med J 2018;24(Suppl 3):S45-7
HMRF project number: 02130156

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This summary is based on studies first reported in: (1) Cheng YKY, Lu J, Leung TY, Chan YM, Sahota DS. Prospective assessment of the INTERGROWTH-21 and WHO Estimated Fetal Weight Reference Curve. Ultrasound Obstet Gynecol 2017 Apr 28. doi: 10.1002/uog.17514. [Epub ahead of print]. (2) Cheng YK, Law LW, Leung TY, Chan OK, Sahota DS. Soluble fms-like tyrosine kinase-1, placental growth factor and their ratio as a predictor for pre-eclampsia in East Asians. Pregnancy Hypertens 2018;11:61-5.

Introduction

Pre-eclampsia is a systemic disease that involves multiple organs and can cause significant maternal and perinatal morbidity and mortality.[1] Pre-eclamptic women are at risk of complications such as eclampsia, stroke, liver and renal failure, pulmonary oedema, and disseminated intravascular coagulopathy. Pre-eclampsia is often associated with iatrogenic preterm delivery that has short- and long-term consequences for the foetus/neonate during the antenatal and postnatal period, as well as in the child's later life. Early-onset pre-eclampsia is associated with foetal demise, a fourfold increase in the risk of intrauterine growth retardation, and an increased risk of cardiovascular disease, hypertension, and diabetes in adult life.[2]

Pre-eclamptic patients have decreased levels of angiogenic factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), and increased levels of anti-angiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1). These angiogenic and anti-angiogenic proteins are produced by placental trophoblasts. Angiogenic factors promote angiogenesis by interacting with members of the VEGF receptor family, whereas anti-angiogenic factors counteract angiogenic effects by binding with circulating VEGF and PlGF, thereby preventing the activation of membrane-bound receptors. The ratio of the serum or plasma concentrations of anti-angiogenic to angiogenic factors, specifically the sFlt-1 to PlGF ratio, has been shown to increase in women with pre-eclampsia and can be used to rule in or out pre-eclampsia.[3]

The study of Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia (PROGNOSIS) indicated that the ratio of concentrations of anti-angiogenic to angiogenic factors had both a high positive and a negative predictive value.[3] Nonetheless, subjects in the PROGNOSIS study were predominantly of European or Afro-Caribbean origin. Differences in body size between Asians and Europeans and Afro-Caribbeans may affect the level of specific markers of pre-eclampsia secondary to haemo-dilution and haemo-concentration effects. For example, the average weight of Chinese women who attend the Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, for Down's syndrome screening is 55 kg—approximately 10 kg lighter than Caucasian and Afro-Caribbean women at the equivalent gestational period. Currently, the ratio of concentrations of anti-angiogenic to angiogenic factors is not adjusted for maternal characteristics such as weight, even though the evidence from Down's syndrome screening indicates that serum concentrations are reduced in heavier women who have a greater blood volume.

Methods

This prospective cross-sectional cohort study aimed to determine the levels of sFlt-1 and PlGF in Chinese

women with a spontaneously conceived singleton pregnancy who presented between April 2015 and April 2016 at the Prince of Wales Hospital. The details of patient recruitment, socio-demographic characteristics, pregnancy characteristics, and pregnancy outcomes have been reported.[4,5] In brief, blood samples were randomly collected from 953 women between 20 and 39 weeks of gestation.[4,5] Serum concentrations of sFlt-1 and PlGF were determined by using an electrochemiluminescence immunoassay (Cobas e411; Roche Diagnostics, Rotkreutz, Switzerland).[5]

Serum levels of sFlt-1 and PlGF were measured in 81 women who had been admitted to hospital for high blood pressure with suspected pre-eclampsia. A detailed description of the pregnancy characteristics and pregnancy outcome has been reported.[5] In brief, 34 (42%) women were confirmed to have pre-eclampsia and 52 (64%) were preterm admissions.[5] A cut-off value of ≥38 or <38 for the sFlt-1 to PlGF ratio was used to rule in or out pre-eclampsia, respectively.[3]

Gestation-specific references for sFlt-1, PlGF, and their ratio were constructed using the R statistical software package and the generalised additive models for location, scale, and shape.

Results

The gestational temporal relationship of sFLt-1, PlGF, and their ratio in the cross-sectional cohort are shown in the Figure. The best-fit models indicated that median, coefficient of variation, and skewness for sFlt-1 and PlGF were dependent on gestation.[5] Both sFlt-1 and PlGF were significantly dependent on maternal weight after correcting for gestation.[5]

A detailed description of the clinical utility of the sFlt-1 to PlGF ratio in the 81 women with suspected pre-eclampsia has been reported.[5] In summary, the 34 women with pre-eclampsia had a significantly higher median sFlt-1 to PlGF ratio than the women without pre-eclampsia, of whom 26 (76.5%) had an sFlt-1 to PlGF ratio of ≥38 at the time of admission.

Discussion

In summary, sFlt-1, PlGF, and sFlt-1 to PlGF ratio exhibited a quadratic relationship with gestation, and both biomarkers were dependent on maternal weight after adjusting for gestation.[5] Zeisler et al[3] reported that a sFlt-1to PlGF ratio of <38 had a negative predictive value of 99.3%. In this study, in the 81 Chinese women admitted with suspected pre-eclampsia, the negative predictive value was only 78.4%.[3,5]

Reliable prediction of pre-eclampsia within specific time intervals using the sFlt-1 to PlGF ratio in women who are symptomatic is important. Accurate prediction enables clinicians to decide who can be managed expectantly and who requires immediate delivery because of poor or rapidly deteriorating maternal or foetal conditions. In some cases, pre-eclampsia can be ruled out and unnecessary hospital admission can be avoided. The sFlt-1 to PlGF ratio is recommended as a complementary test to the traditional measurements of blood pressure and proteinuria, as both provide only limited information about the course and severity of the disease.

The clinical utility of the sFlt-1 to PlGF ratio was assessed in the 81 pregnancies only. We were therefore unable to determine whether a high ratio was associated with immediate delivery and disease severity. We were also unable to assess whether the

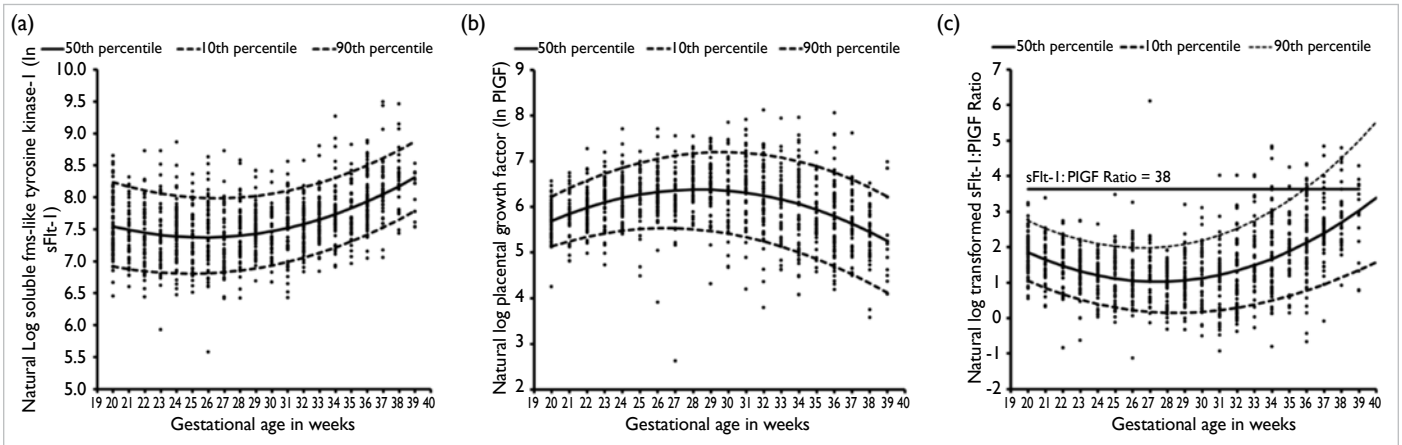

FIG. Relationship of gestational age with natural log values of (a) concentration of soluble fms-like tyrosine kinase-1 (sFlt-1), (b) concentration of placental growth factor (PlGF), and (c) sFlt-1 to PlGF concentration ratio, showing the cut-off value to rule in or out pre-eclampsia.[3]

ratio was predictive of adverse perinatal outcomes such as poor Apgar score, cord arterial pH at delivery, or whether it was correlated with uterine artery Doppler indices. An sFlt-1 to PlGF ratio of >655 has been reported to be able to identify women at risk of preterm delivery before 34 weeks of gestation and predict poorer perinatal outcome in women with clinical signs of pre-eclampsia.[6]

Given that the biomarkers of sFlt-1 and PlGF are dependent on gestational age and maternal weight, and that Chinese women are smaller in size, it remains questionable whether the cut-off ratio of 38 can be applied equally in Chinese populations. Further large-scale studies to assess the clinical utility of the two biomarkers in Chinese populations are needed to provide optimal management and to avoid unnecessary iatrogenic delivery.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#02130156). We thank the members of the foetal medicine team, midwives, and research assistants at the Prince of Wales Hospital.

Ethical Approval

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (CREC – 2014.507).

Declaration

The authors have no conflicts of interest to disclose.

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